Comparaison de la reproduction sexuée et du recrutement des coraux scléractiniaires entre un récif tropical (La Réunion) et subtropical (Afrique du Sud) du sud-ouest de l'océan Indien

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Résumé

Abstract

In the context of climate change, coral reproduction and recruitment are key processes which can ensure the renewal and recovery of reefs. Yet the effects of climate change and global warming on these processes is poorly known and may vary with latitude. This project aimed to compare the sexual reproduction and recruitment of two reef-building corals at two latitudes in Reunion and South Africa to ascertain how fecundity, larval survival and recruitment rate varied for similar species depending on latitude and environmental conditions. It involved the sampling of coral using scuba-diving, histological analysis, the deployment of recruitment tiles and aquarium experiments.

The reproductive features differed little between the two coral species studied in Reunion and South Africa, viz. *Acropora austera* and *Platygyra daedalea*. This consistency in coral reproduction suggests that slight differences in the environmental parameters do not affect coral reproductive capacity. It may also indicate that some corals may have limited capacity to adapt their reproduction in different environments. Fecundity in *A. austera* was lower in Reunion (6.9±0.3 oocyte per polyp) than in South Africa (9.9±0.3 oocyte per polyp). This may be due to the occurrence of more stressful environmental conditions at Reunion (high levels of pollution relative to near pristine conditions on South African reefs) that may affect the coral reproductive output. Despite being a slow-growing coral, *P. daedalea* invested a large amount of energy into reproduction and produced up to 60 oocytes per polyp. Its fecundity, in addition to a more stress-tolerant capacity, may enhance its chance of survival in the face of climate change.

The recruitment rate was high in South Africa (1054 recruits m^2 $year^{-2}$) and close to the value observed on the Great Barrier Reef. In Reunion, the average recruitment rate (279 recruits m^2 $year^{-2}$) ranged between values observed on other tropical fringing reefs. The South African reefs seemed, therefore, to be more suitable for coral settlement, despite being at the limit of coral environmental threshold. Apart from pocilloporids, acroporids were the most abundant in South Africa while it was poritids in Reunion. The recruit composition on tiles seemed to reflect the taxonomic composition of the adult assemblages. This pattern suggests that the recruitment on these reefs may rely mainly on the local larval input and/or that the post-settlement mortality is high in recruits not adapted to the local conditions on the reefs. The reefs might therefore manifest slow recovery following the mortality of adult colonies. No

clear difference in coral recruitment rate or diversity was evident between the No-take Areas (NTAs) and exploited reefs of Reunion. This could be due to the young age of the MPAs (enforced in 2007) and/or the limited size of the NTAs (5% of the total MPA surface) that may have limited influence on coral recruitment. Recruitment rate was, in fact, lowest in the northern NTA (SAT) where reef degradation and signs of eutrophication have been reported since the 1980s. Conversely, other sites outside the NTAs yielded high recruitment rates; there may therefore be a need to re-evaluate the status of NTAs within the MPAs.

Gametogenesis in the two species studied was strongly correlated with change in seawater temperature in Reunion and South Africa. Variation in temperature may therefore affect the synchronicity and timing of reproduction in these corals and have major consequences on their fertilisation and reproductive success. In addition, increased seawater temperature strongly affected coral development in aquarium by reducing the larval pre-competency period. A shorten benthic phase may therefore favour localised recruitment and lead to greater reef isolation. This may have serious consequence for reef recovery following damage.

Remerciements

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Chapter 1:

Comparison of reproduction in two reef-building corals in the south Western Indian Ocean : *Acropora austera* and *Platgyra daedalea*

Introduction

Global warming is recognised as one of the most severe threat to coral reefs. Subtropical (25-30°) and high-latitude reefs (>30°) may be protected from thermal stress and could serve as a refuge against high temperature (Amat & Bates 2003; Riegl & Piller 2003; Lybolt et al. 2011). Nevertheless, they lie at the limit of the environmental thresholds for coral and are marginal in terms of temperature, light and aragonite saturation (Kleypas et al. 1999). These reefs were first believed to be unsuitable for coral development and reproduction (Wells 1957; Veron et al. 1974). Important coral communities have, however, developed up to 32°S and showed active sexual reproduction, thereby proving that they are not pseudo-populations (Shlesinger & Loya 1985; Babcock et al. 1994; van Woesik 1995; Harii et al. 2001; Wilson & Harrison 2003; Fellegara et al. 2013). The unusual environmental conditions occurring at the limit of coral development have, however, proven to interfere with coral reproduction. **Erreur ! Source du renvoi introuvable.**Reduced or fluctuating water temperatures are usually associated with asynchronous split spawning that may diminish the rate of fertilisation (Hayashibara et al. 1993; Wilson & Harrison 2003; Nozawa et al. 2006). Low seawater temperature tends to slow down the rate of gamete maturation and delays the spawning period (Dai et al. 1992; Harii et al. 2001; Wilson & Harrison 2003; Massé et al. 2013b). While only few studies have compared the reproductive traits of corals between a tropical and a marginal reef, no study has investigated this aspect in the Western Indian Ocean. Furthermore, little is known on the reproductive effort and coral fecundity in a marginal environment.

Spawning in corals is known to be controlled by a combination of environmental factors that ensure the synchronisation of gamete release between colonies and a high rate of fertilisation (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Oliver et al. 1988). Seawater temperature, solar radiation, lunar phase and diurnal light cycle are considered proximate cues, which operate at successively finer time scales (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Oliver et al. 1988; Penland et al. 2004). Nevertheless, the timing of synchronous spawning varies geographically (see reviews in Harrison & Wallace 1990; Richmond & Hunter 1990) and other environmental factors (or combination of factors) have to be considered (Harrison and Wallace 1990). For example, spawning occurred in the month prior to the period of heaviest rainfall in *Montastrea annularis* of Jamaica suggesting that the coral may adapt the timing of spawning to the local environmental conditions (Mendes & Woodley 2002b). The delay in seasonal variation of certain environmental parameters

between the tropical and subtropical reefs may drive different pattern in the timing of spawning and the breeding season.

The South African reefs lie at the southernmost distribution of coral along the East African coast (27-28°S). In contrast, the reefs of Reunion occur in tropical water (21°S). They provide therefore a good opportunity to compare the coral reproduction between two different reef environments. Two species, *Acropora austera* and *Platygyra daedalea,* characterised by different life-history traits, were chosen to assess for difference in the (1) gamete development, (2) fecundity and (3) spawning between South Africa and Reunion. The influence of seawater temperature, light intensity and rainfall on coral gametogenesis and spawning were assessed at the two localities.

Materials and methods

1. Study sites

Coral reproduction was studied at two sites in South Africa and Reunion to ascertain its variability at the local scale. The sampling was carried out at 10-15 m deep at Five-mile (FMR, 27°29'28.68"S, 32°41'37.44"E) and Two-mile Reef (TMR, 27°31'22.56"S, 32°41'10.86"E) in South Africa, and at la Saline (SAL, 21°06'41.6"S, 55°14'59.2"E) and St Leu (SLE, 21°10'55.12"S, 55°17'1.96"E) in Reunion (Fig. 1).

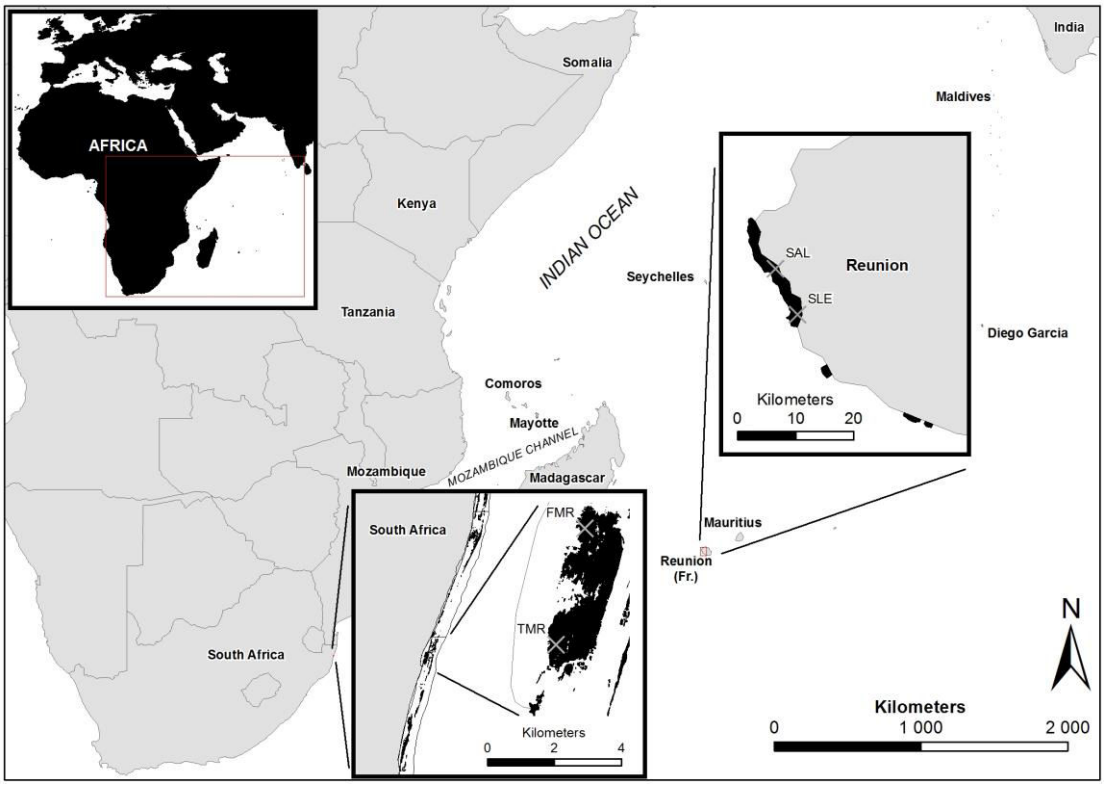

Figure 1: Location of the experimental sites for the survey of coral reproduction in South Africa and Reunion. TMR: Two-mile reef; FMR: Five-mile reef, SAL: la Saline; SLE; St Leu.

2. Sampling

Their identification of *Acropora austera* and *Platygyra daedalea* was verified using genetic prior sampling (Montoya-Maya 2013). The same sampling procedure was used to follow gamete development in South Africa and Reunion. Monthly sampling was carried out simultaneously (±7 days, Table 1) in the two countries, around full moon, from October 2010 to October 2012 (two years, Table 1). Sampling was intensified when possible around the predicted date of coral spawning. Seven samples of both species were collected each month.

Only large (mature) colonies of *A. austera* (>15 cm in diameter) and *P. daedalea* (>18 cm in diameter) were selected for sampling. Branches of *A. austera* were broken as close as possible to their base using a screw-driver. In *P. daedalea*, sampling of the growing edge was avoided to ensure that only fecund polyps were harvested (Szmant 1991; Soong & Lang 1992; Sakai 1998). A stainless steel tube and a hammer were used to collect cores of 21 mm diameter close to the centre of these massive colonies. This diameter was chosen to limit damage to the colonies, yet yielded sufficient tissue for analysis (~20 polyps). The cores and the branches of two coral species were fixed in formalin 4% for at least 48 h before processing.

Table 1: Dates of coral sampling in South Africa and Reunion for the reproduction survey. FM: full moon; n/a: no data.

		South Africa			Reunion	
	Date of sampling	Date of FM	FMR	TMR	SLE	SAL
YEAR 1	2010					
	Sep	23/09	n/a	9/09	22/09	22/09
	Oct	23/10	18/10	n/a	6/10 and 21/10	6/10
	Nov	21/11	n/a	10/11	08/11 and 19/11	12/11
	Dec	21/12	9/12	9/12	3/12 and 17/12	3/12
	2011					
	Jan	19/01	n/a	n/a	21/01	21/01
	Feb	18/02	9/02	10/02	21/02	21/02
	Mar	19/03	02/03 and 09/03	2/03 and 10/03	17/03	17/03
	Apr	18/04	14/04	14/04	n/a	n/a
	May	17/05	n/a	n/a	n/a	n/a
	Jun	15/06	n/a	n/a	18/06	18/06
	Jul	15/07	14/04	14/07	n/a	n/a
	Aug	13/08	n/a		12/08	12/08
YEAR 2	Sep	12/09	14/09	14/09	13/09	n/a
	Oct	12/10	12/10	12/10	12/10	n/a
	Nov	10/11	16/11	16/11	10/11	17/11
	Dec	10/12	13/11	13/11	12/12	13/12
	2012					
	Jan	09/01	11/01	11/01	03/01 and 19/01	19/01
	Feb	07/02	07/02	07/02	09/02 and 16/02	16/02
	Mar	08/03	14/03	14/03	07/03	8/03
	Apr	06/04	17/04	17/04	09/04	09/04
	May	06/05	25/05	25/05	n/a	n/a
	Jun	04/06	14/09	14/09	12/06	12/06
	Jul	03/07	n/a	n/a	n/a	n/a
	Aug	2/08	n/a	n/a	12/08	12/08

3. Histology

The detailed description of gamete development and reproductive condition of the corals was ascertained using histology. The coral samples were decalcified using a gradient of hydrochloric acid (HCl 1-3%) mixed with a buffer EDTA solution (1 $mg.l^{-1}$). They were then dehydrated in an alcohol series, cleared using xylene and embedded in paraffin wax (Peters & Price 2008). Twenty serial cross-sections of 8 µm thick were cut at intervals of 40-80 µm using a microtome, and stained with eosin and Harry's haematoxylin (Peters & Price 2008). In *A. austera*, the tissue was sectioned longitudinally and a fragment of 2x3cm (~30 polyps) was cut avoiding the tips and the base of the branch which are reported to be less fertile (Wallace 1985). Photographs of gametes from 10 randomly-selected sections were taken under the microscope at 100x and 200x magnification using AxioVision 5 software (2006-2010 Carl Zeiss MicroImaging GmbH).

Gamete development was classified according to the cell size and morphology criteria of Szmant-Froehlich (1980). Further descriptions of gamete development specific to acroporid and faviid corals were obtained from Vargas-Ángel and co-authors (*2006, Acropora cervicornis*) and Weil and Vargas (2010, *Diploria* spp). The proportion of gametes per development stage was noted and size measurements were obtained from seven randomly selected gametes per development stages. Only oocytes with a visible nucleus were measured (i.e. those centrally sectioned) for the estimation of oocyte size. The mean diameter of gametes was calculated as an average of the maximum and minimum diameters measured at right angles. Samples, which had less than three oocytes and contained no spermary in the sections examined, were considered as empty.

4. Fecundity

Fecundity measurements were obtained from dissected decalcified material in seven colonies per species collected prior to spawning in 2012 (i.e. in February 2012 for South Africa and March 2012 for Reunion). The size and number of oocytes were estimated in ten polyps per colonies that were dissected under a stereo microscope to expose their mesenteries, and photographed using AxioVision 5 software. The oocyte size was measured as described in the previous paragraph. To estimate the overall reproductive output, the fecundity index was calculated by multiplying the mean oocyte size and number per polyp or mesentery, divided by 100.

5. Monitoring of spawning

Scuba-diving at night on the reef is difficult, so coral spawning was monitored *in situ* on the reef flat of Reunion (St Leu) and completed with aquarium observation and histological analyses of samples collected close to the predicted date of spawning. The prediction of spawning in South Africa and Reunion was based on the spawning date known for other coral families. In South Africa, coral spawning was inferred to occur between January and February in histology samples of *P. verrucosa* and several soft corals species (Schleyer et al. 1997; Kruger et al. 1998; Kruger & Schleyer 1998). In Reunion, spawning in *Acropora* spp. occurred usually 2-4 days after the full moon of October or November (Appendix 1**Appendix 1**).

A total of 40 night-dives were carried out from September 2010 to January 2012 on the St Leu reef flat with the help of 60 volunteers (Table 2). Every night, information about the time, location and species involved in spawning was noted.

Table 2: Sampling schedule for the *in situ* observations of coral spawning in Reunion. FM: full moon; NM: new moon.

Year	Month	Day	Moon phase	Total number of night
2010	Sep	25, 26	FM +2- 3	2
2010	Oct	23, 24, 25, 26, 27, 28, 29	FM- FM+6	7
2010	Nov	21, 22, 23, 24, 25, 26, 27	FM- FM+6	7
2011	Oct	13, 14, 15, 16, 17, 18, 19	FM +1- 7	7
2011	Nov	13, 14, 15, 16	FM +2- 5	4
2011	Dec	11, 12, 13, 14, 15, 16	FM +1- 6	6
2012	Jan	9, 12, 13, 15, 16, 17	FM- FM +7	6
2012	Feb	25	NM +3	1
			TOTAL	40

Additionally, seven to 10 colonies of *A. austera* and *P. daedalea* were collected in South Africa (Two-mile reef, TMR) and Reunion (St-Leu, SLE), a few days before the predicted date of spawning in 2011 and 2012 for aquarium monitoring (Appendix 2). They were placed in separate, open-water aquaria with a photoperiod 8h-16h light-dark in the ORI research aquarium and the aquarium of La Reunion and maintained at ambient temperature (26°C and 27°C in South Africa and Reunion respectively). Spawning was monitored from dawn over ten nights each month. When possible, samples were collected from *in situ* colonies to ascertain the occurrence of spawning on the reefs.

6. Environmental variables

The timing of gamete maturation and spawning in the two studied species was correlated with water temperature, light intensity, rainfall and lunar phase to investigate the influence of these parameters on coral reproduction. In South Africa, seawater temperature as hourly means of minute-interval measurements were recorded at 18 m on Nine-mile Reef (27°24'53.7''S; 32°43'35.8''E) using an underwater temperature recorder (Hugrun UTR probe) reading to a minimum accuracy of 0.02°C. In Reunion, hourly data were obtained from a temperature sensor (Sensor 1T3141, accuracy of 0.01°C) deployed at SLE from the 8th October 2010 to the 10th October 2011 (ARVAM, unpublished data). Data estimated from satellite imagery Coral Reef Watch program (NOAA 2012) were used to complete this dataset and covered the second year of study. They proved to provide a good estimation of seawater temperature as no significant difference was found between the *in situ* and satellite measurements during year 1 (October 2010-October 2011, t-test, $p>0.05$, Appendix 3). Light intensity (mJ m^{-2} day^{-1}) and rainfall (mm day^{-1}) were obtained from the NASA's Fast Longwave And SHortwave Radiative Fluxes project (FLASHFlux, http://flashflux.larc.nasa.gov/). Lunar phase at the time of spawning in the two species was obtained from the South African Astronomical Observatory (www.saao.ac.za) for the two study regions.

7. Statistical analyses

Significant differences in gamete size and fecundity were ascertained using One-way ANOVA performed on Statistica 10, '1984-2011, Statsoft, Inc). To meet the test assumptions, the data were transformed as (x+1)^0.1, where x was the variable to test, and their homogeneity was verified with the Levene's test. All measurements were found to be homogenous (Levene's tests, $p>0.05$). Gamete maturation was estimated by looking at the increase in gamete size over time. It was best estimated by linear regressions, which were compared using the F-test for unequal variance, between study sites, regions and year. These graph analyses were done using Graphpad prism 5 (1992-2010 GraphPad Software, Inc). The influence of environmental parameters on gamete size and time of spawning was ascertained using Spearman's product moment correlation. The r^2 and slope of the regression were calculated for each correlation and their significance at $\alpha = 0.05$ was determined according to the critical values for the Pearson correlation.

Results

Acropora austera

1. Reproductive mode

A total of 392 colonies (186 and 206 colonies in South Africa and Reunion respectively) of *A. austera* were analysed over the two years study. *A. austera* was hermaphroditic in South Africa and Reunion i.e. contained male and female gametes at maturity (Fig. 2). In rare cases, female or male colonies were observed in the samples. This concerned a total of three and two female colonies in South Africa and Reunion respectively, and two male colonies in South Africa. No developing embryos or planulae were observed in the histological samples over the two years of study proving that this coral was not a brooder but a broadcast spawner. This mode of reproduction was confirmed by the observation of gamete release in aquarium. No differences in the arrangement of gonads or morphology of gametes during development were observed between colonies collected in South Africa and Reunion. The gonads of *A. austera* occurred within the mesogloea of the eight complete mesenteries. The female gonads developed in the short and long pairs of lateral mesenteries and appeared as single strings of oocytes (Fig. 2). Spermaries occurred in the directive mesenteries of the polyp and were cone-shaped.

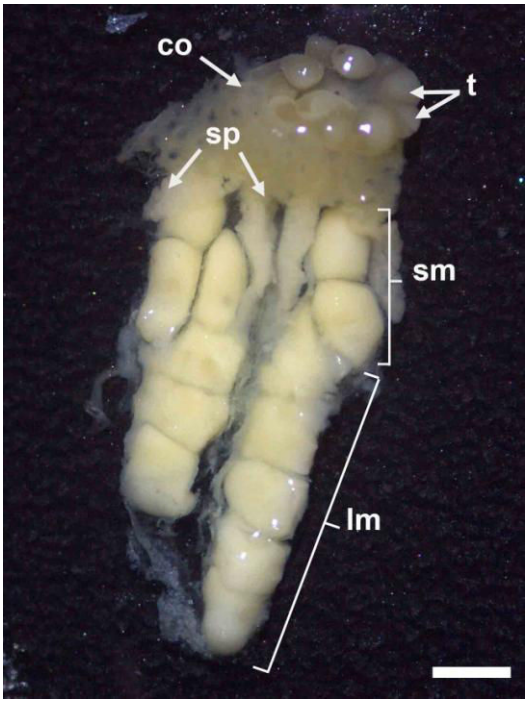

Figure 2: Dissected polyp of *Acropora austera*. co, coenosteum; lm, long mesentery; sm, short mesentery; sp, spermary; t, polyp tentacles. Scale bar = 500 µm.

2. Gametogenesis

Four gametogenic stages were identified in *A. austera* during oogenesis and spermatogenesis. They are illustrated in Figures 3 and 4. The mean size of each developmental stage is shown in Table 3.

2.1.Oogenesis

Stage I oocytes were enlarged interstitial cells of 20-110 µm (Fig. 3A). They were characterised by a prominent nucleolus surrounding by a thin nucleus. Together, the nucleolus and nucleus occupied one third of the egg volume. Yolk granules were observed in their cytoplasm from the beginning of oogenesis, indicating the early initiation of vitellogenesis. In Stage II oocytes, the cytoplasm had extended and the nucleus was thickened (Fig. 3B). This later occupied approximately one fifth of the egg volume. Stage II oocytes were 90-210 µm. Their nucleus and cytoplasm continued to increase in size until they reached 180-310 µm, which corresponds to Stage III oocytes (Fig. 3C). Stage IV was mature oocyte of 280-610 µm in size. Their cytoplasm was filled with lipid vesicles and the nucleus had begun to migrate from the centre towards the periphery of the oocyte (Fig. 3D). Prior to spawning, they were irregularly shaped, probably due to limited space in the mesentery. No zooxanthellae was present in Stage IV oocytes. Pink coloration in mature oocyte was observed in fresh sampled collected at TMR and FMR on the 8 February 2012, i.e. seven days before spawning.

2.2.Spermatogenesis

Stage I spermaries consisted of individual primordial cells enveloped in the mesogloea of the mesentery (Fig. 4 A). They were circular and ranged in size from 15-50 µm. In early stage I spermaries, the internal cell structure was not discernible and was difficult to distinguish from the mesogloea. From Stage I to Stage II, the primordial spermary cells grew in size and the spermatocytes became visible at the periphery of the spermatogonial wall (Fig. 4 B). Stage II spermaries ranged between 25-60 µm in size. Stage III spermaries (40-140 µm) consisted of numerous spermatids, giving the gonad a granular appearance (Fig. 4 C). The spermatids were mostly concentrated at the periphery of the spermary and arranged around a central lumen. Stage IV spermaries contained mature sperm and were large-bodied and intensively stained (60-190 µm, Fig. 4 D). The spermatogonial wall was reduced to a thin membrane. Since the spermaries were extremely dense, the distinctive characteristics of the spermatozoa were not visible.

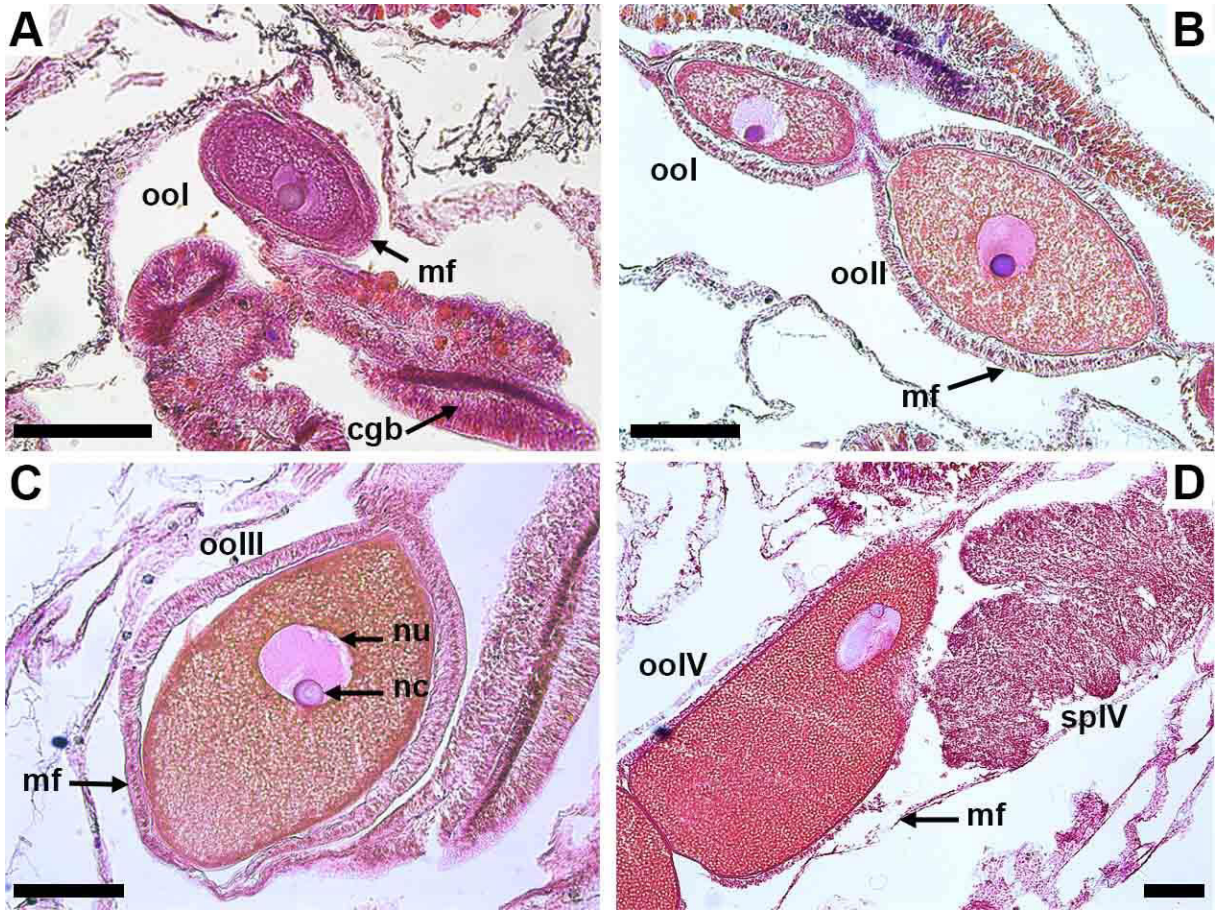

Figure 3: Oocyte maturation in *Acropora austera*. A, Stage I oocyte showing the prominent nucleolus. B, Stage I and II oocytes developing in the same mesentery. C, Stage III oocytes. D, Mature Stage IV oocyte adjacent to Stage IV spermary. Cgb: cnidoglandular band; mf: mesenterial filament; nc, nucleolus; nu, nucleus; ooI, ooII, ooIII, ooIV: oocytes Stage I, II, III, IV respectively; spIV, spermary Stage IV. Scale bars are 100 µm.

3. Seasonality of gametogenesis

The seasonality in oogenesis and spermatogenesis is shown in Figures 5 and 6. It occurred at the same time of the year in South Africa and Reunion, despite small variations (± 1 month) between study sites and years. The colonies manifested an annual gametogenic cycle of 5-6 months. Overall, oogenesis was initiated at the end of the winter in September to October. Spermatogenesis was delayed compared to oogenesis and started on average 2-3 months later. Oogenesis and spermatogenesis ended simultaneously with spawning in February or March as inferred by the disappearance of mature gametes in the samples (see below section 3.1). Several colonies of *A. austera* lacked gametes during gametogenesis in South Africa and Reunion.

3.1.Seasonality in oogenesis

Oogenesis was initiated with the appearance of Stage I oocytes in the polyp mesentery (Fig. 5). It started in September at TMR and SLE and in October at FMR and SAL. In one exception (1 colonies over the seven sampled), the early oocytes (Stage I) were observed in August in Reunion (SLE). Oocyte development was synchronised between study sites but not between the two regions. In year 1, the oocyte development was slightly faster in Reunion than in South Africa as shown by the dominance of Stage III oocytes in December in Reunion while numerous Stages I and II were still observed in South Africa. In year 2, the opposite trend was observed. Stages III and IV oocytes were first observed in November in South Africa while they appeared in January in Reunion.

1.1.Seasonality in spermatogenesis

In year 1, spermatogenesis commenced simultaneously at FMR, TMR, and SAL in December Fig. 6. It was, however, initiated two months earlier at SLE. In year 2, the initiation of spermatogenesis was synchronised between the study sites but not between the regions. The first spermaries appeared in November at FMR and TMR and one month later at SAL and SLE. The occurrence of spermaries was positively correlated with the increasing numbers of Stage III ova ($r^2 = 0.94$) in South Africa but this correlation was weak in Reunion ($r^2 = 0.52$). It was also negatively correlated with the disappearance of Stage I oocyte in South Africa ($r^2 = 0.74$) but this was not observed in Reunion. Spermary development was highly synchronised between colonies, with most spermaries being at the same stage of differentiation within the polyp mesenteries. As noted for the oocyte development, the spermary development occurred earlier in Reunion than in South Africa in year 1 but the opposite trend was observed in year 2. In year 2, Stage III and IV spermaries were dominant in the polyp in January and February, while it was in February and March in Reunion. Overall, the duration of spermatogenesis was and lasted 3-4 months in South Africa and Reunion, except at SLE where it lasted up to 5 months in year 1.

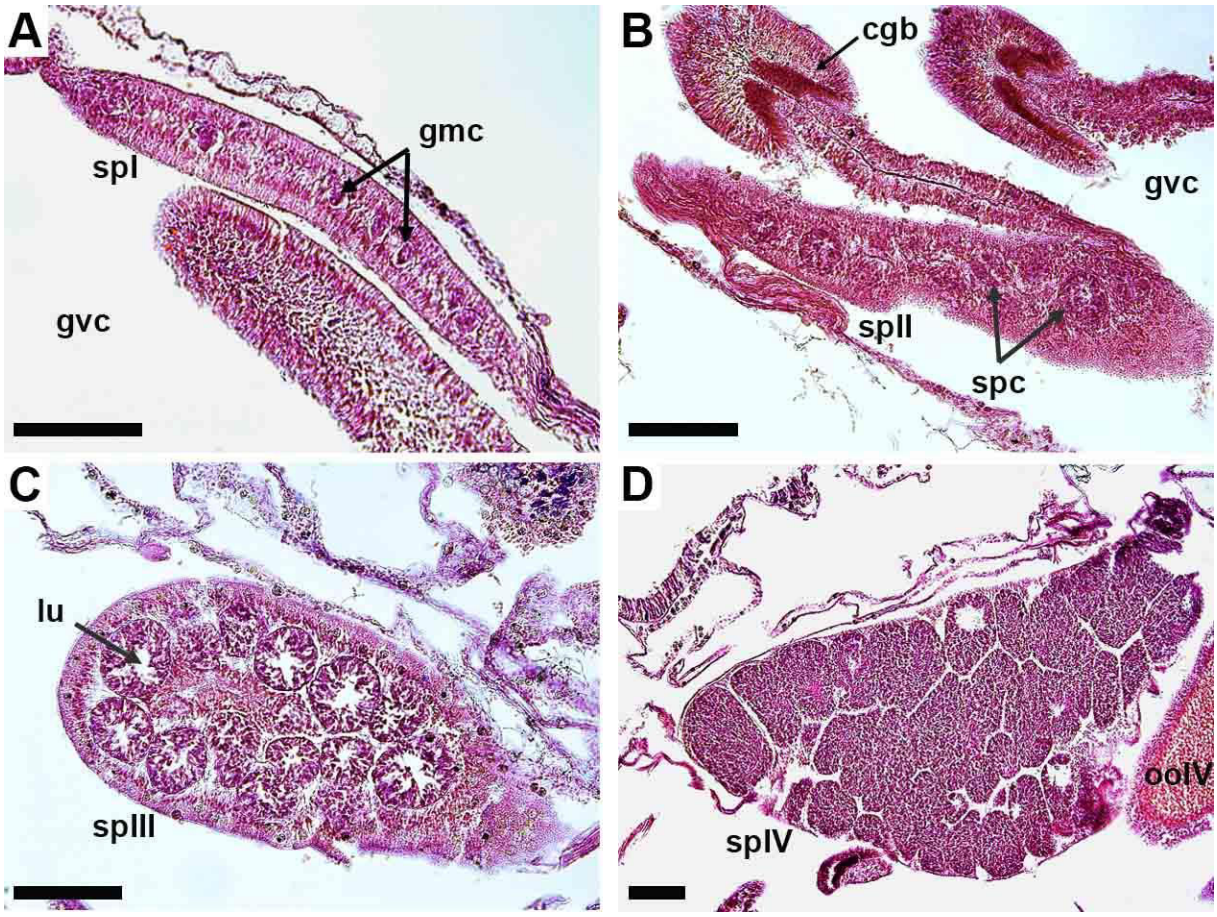

Figure 4: Spermary maturation in *Acropora austera*. A, Stage I spermary consisting of individual primordial cells. B, Stage II spermaries showing spherical spermatocytes. C, Stage III spermary characterised by a lumen. D, Mature Stage IV spermary. Gmc: germ cell; gvc:gastrovascular cavity; lu: lumen; ooIV, oocyte Stage IV; spc: spermatocyte; spI, spII, spIII, spIV: spermary Stages I, II, III, IV respectively. Scale bars are 100 µm.

Table 3: Mean oocyte and spermary sizes in histological preparation of *Acropora austera* according to the stage of maturation (I, II, III, IV) from Reunion and South Africa.

		Mean size (sd) µm		
		All sites	**South Africa**	**Reunion**
Oocyte	**Stage I**	74.87 (20.46)	73.26 (3.74)	76.48 (20.49)
	Stage II	143.04 (28.21)	140.96 (20.54)	144.74 (26.68)
	Stage III	239.71 (29.15)	238.81 (24.45)	239.63 (32.09)
	Stage IV	369.73 (72.69)	379.77 (72.88)	362.19 (71.76)
Spermary	**Stage I**	28.26 (7.50)	30.29 (7.95)	24.59 (4.96)
	Stage II	41.18 (7.10)	38.01 (7.82)	42.79 (6.76)
	Stage III	76.77 (23.16)	70.32 (15.95)	84.57 (27.78)
	Stage IV	126.62 (33.61)	132.59 (32.18)	100.78 (27.34)

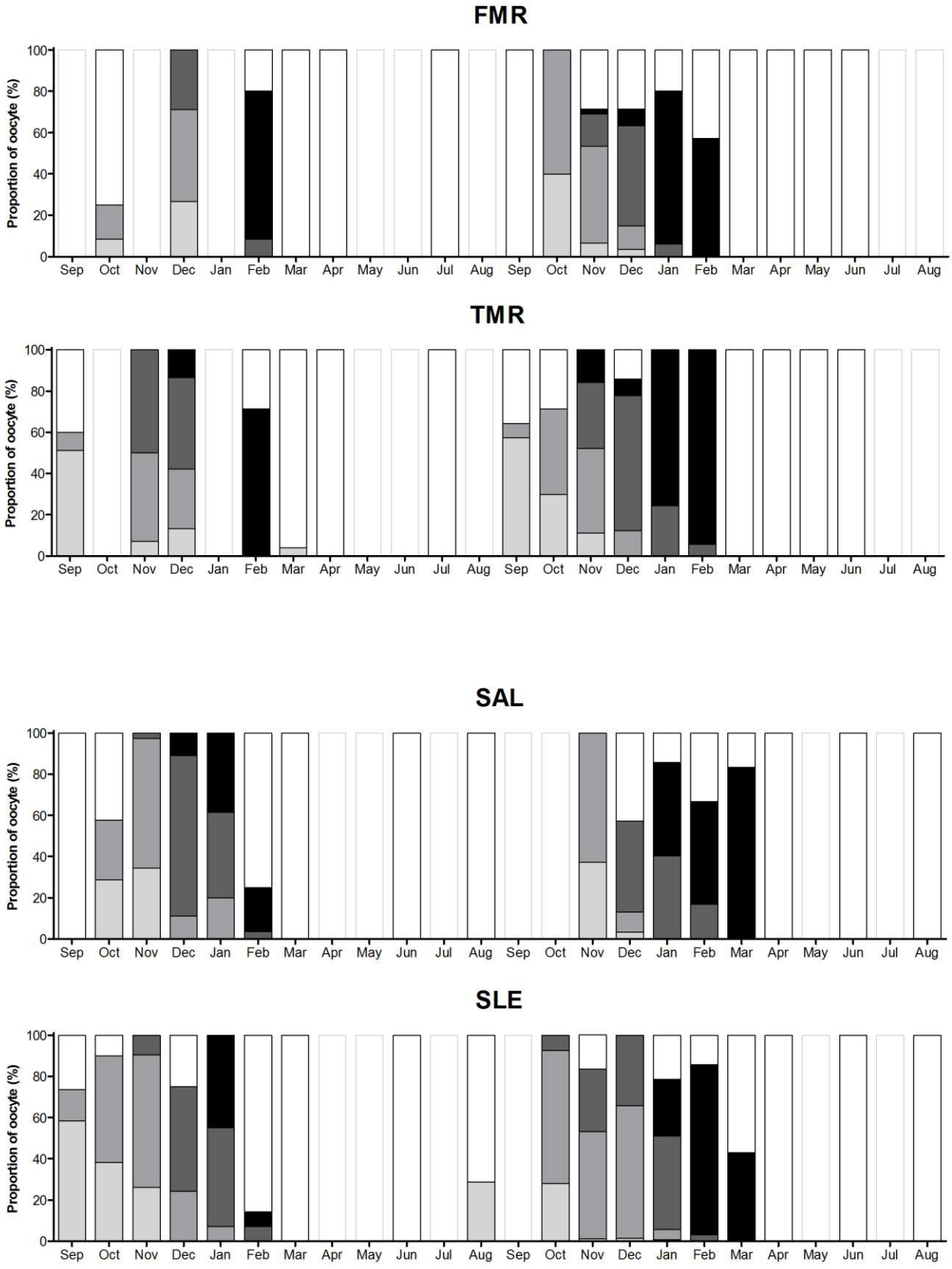

Figure 5: Seasonality in oocyte development in *Acropora austera* at the study sites in South Africa (Two-mile reef, TMR, and Five-mile reef, FMR) and Reunion (la Saline, SAL, and St Leu, SLE). Light grey bars indicate no sampling (unclear).

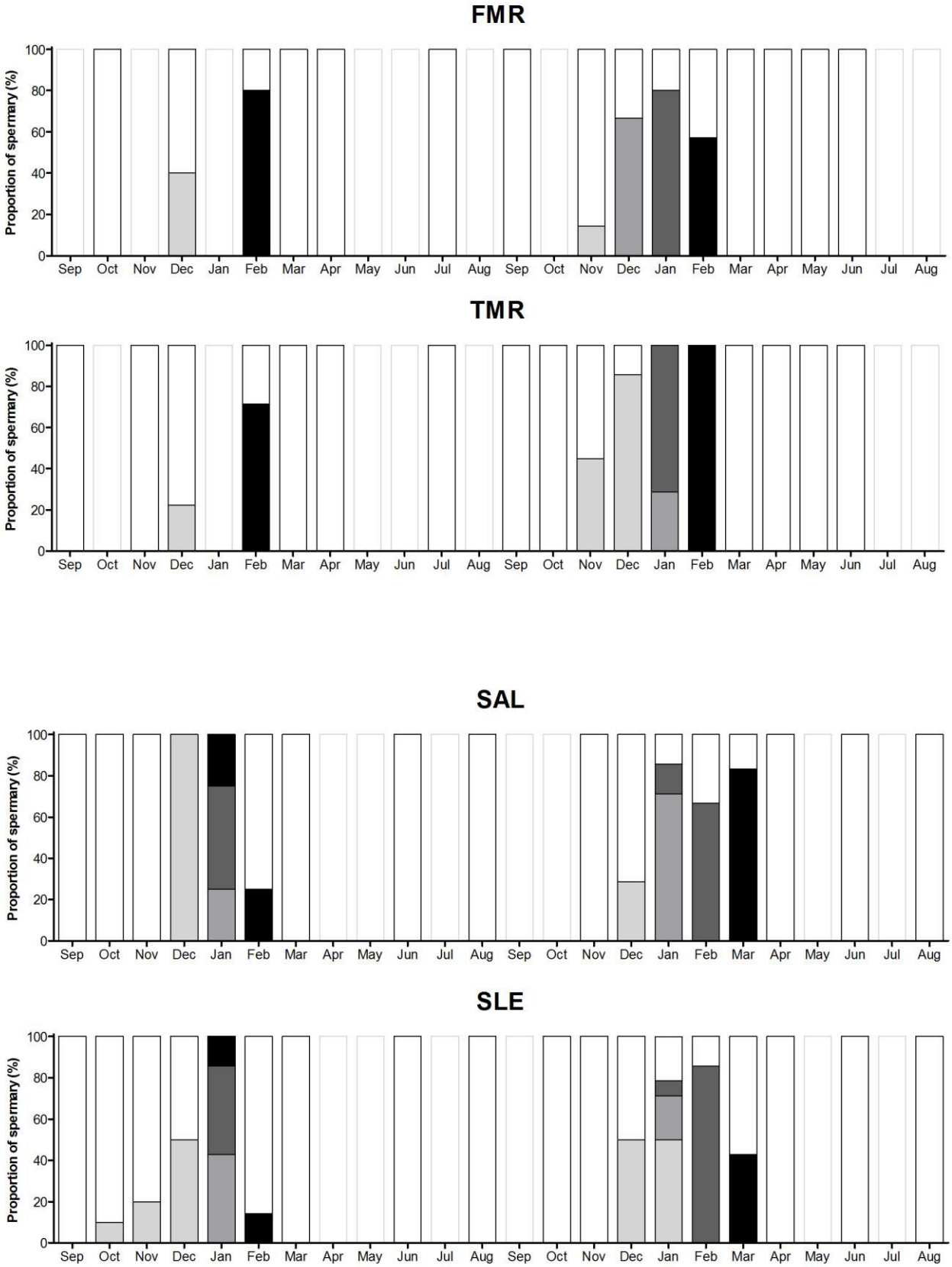

Figure 6: Seasonality of spermary development in *Acropora austera* at the study sites in South Africa (Two-mile reef, TMR, and Five-mile reef, FMR) and Reunion (la Saline, SAL, and St Leu, SLE). Light grey bars indicate no sampling (unclear).

2. Mean gamete size

The mean size of oocytes and spermaries increased over time following the initiation of gametogenesis and did not reach a plateau close to maturity. It was best estimated by linear regressions (r^2 = 0.84-0.96; Fig. 7). The gametes were growing at the same pace in South Africa and Reunion and no significant difference in the elevation of the regression slopes was found between the two regions (F-test, $p>0.05$,

Table **4**) and study sites (F-test, p>0.05). The gamete growth rate was also consistent between year 1 and year 2 (F-test, p>0.05) except at SLE where the growth rate was faster in year 2 than in year 1 (F-test, p>0.05).

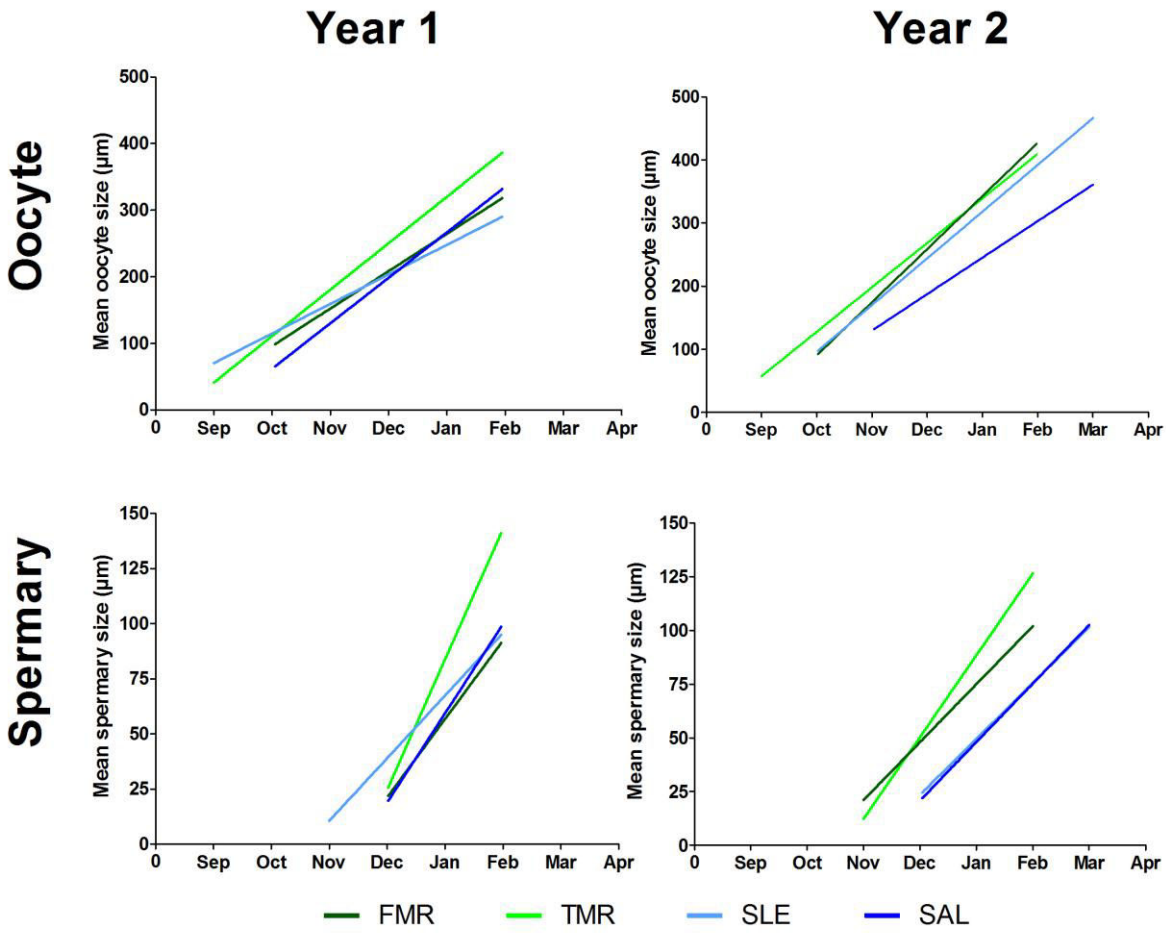

Figure 7: Linear regressions of mean gamete size in *A. austera* per study site. Year 1: September 2010-August 2011; year 2: September 2011-August 2012. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

Table 4: Summary of the F-test values for the comparison of the increase in oocyte size at each study sites. The hyphen means that the test could not be calculated as not enough data were available in 2011. Year 1: September 2010-August 2011; year 2: September 2011-August 2012. Significance: * p<0.05, ** p<0.01, *** p<0.001, ns not significant. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

	Source of variation		F-test		
	Fixed effect	**Random effect**	**F**	**df**	**p**
Oocyte	Between sites	Year 1	1.04	3	ns
		Year 2	0.81	3	ns
	Between years	FMR	1.91	1	ns
		TMR	0.00	1	ns
		SAL	0.32	1	ns
		SLE	5.96	1	*
Spermary	Between sites	Year 1	-	-	-
		Year 2	0.59	3	ns
	Between years	FMR	-	-	-
		TMR	-	-	-
		SAL	1.83	1	ns
		SLE	0.11	1	ns

3. Spawning

3.1.In situ observations

A. austera was not observed spawning *in situ* on the reef flat of Reunion despite the numerous night-dives. Yet, spawning was other in other Acropora spp species (Appendix 4).

3.2.Aquarium observations

Colonies of *A. austera* were observed spawning in aquaria in 2011 and 2012 in South Africa but not in Reunion. In year 1, spawning was observed on the 24th and 25th February 2011 (6-7 nights after full moon, nAFM). During the first night of spawning, three of the ten colonies spawned from 20:30 to 21:10. Bundles of oocytes and sperm were visible in the polyp mouths from 20:00 (Fig. 8A). On 25th February 2011, five more colonies spawned between 22:00 and 22:15 (Figs 8 B and C). The last two colonies showed no spawning activity. In year 2, limited spawning was observed in one of the ten colonies of *A. austera* collected, on the 15th February (8 nAFM) at 22:00. On the 23rd February 2012, oocytes were found floating at the water surface of the aquaria in the morning (8:00) suggesting that some spawning may have occurred on the 22^{sd} February (15 nAFM).

Figure 8: Spawning in *Acropora austera.* A-B: Release of bundles of oocyte and sperm from the polyp mouths. C: Floating bundles at the water surface starting to break apart.

3.3.Histology

In South Africa, the disappearance of the mature gametes in the histological samples of *A. austera* occurred between February and March over the two-year studied and this corresponds with the aquarium observations. In February 2011, respectively one and two colonies respectively at FMR and TMR were empty while the other colonies sampled contained mature gametes. In Reunion, the main disappearance of mature gamete in the samples took place between January and February 2011 and between March and April 2012. Like in South Africa, the gamete release was not simultaneous for all colonies. For example, one and two colonies at SLE and SAL respectively retained gametes in the February 2011, and one of them still contained Stage III oocytes and spermaries (see SLE, Figs 5 and 6). In Year 2, four colonies over the seven sampled at SLE lacked mature gametes in March. This trend was not observed at SAL.

4. Fecundity

A. austera polyps contained significantly more mature oocytes in South Africa than at Reunion (ANOVA, $F = 106.54$, $p<0.01$, Table 5). The size of oocytes was, however, higher in Reunion than in South Africa but this difference was not significant (ANOVA, $F = 0.14$, $p>0.05$). Nevertheless, the combination of the number and size of oocytes (fecundity index) in *A. austera* was significantly higher in South Africa than Reunion (ANOVA, $F = 77.51$, $p<0.01$).

At the local scale, the number of oocyte per polyp was not significantly different within study sites in a same region (Tukey t-tests, $p>0.05$,Table 6), the oocyte size varied however significantly between FMR and TMR (TMR>FMR, Tukey t-test, $p<0.001$) and between SAL and SLE (SLE>SAL, Tukey t-test, $p<0.001$, Table 6). The fecundity index was significantly different between FMR and TMR (TMR>FMR, Tukey t-tests, $p<0.001$) but not between SLE and SAL (Tukey t-tests, $p>0.05$). At SAL, the smaller oocyte size was compensated by a greater number of oocyte per polyp. The correlations between the number and size of oocyte per polyp were weak in South Africa ($r^2 = 0.01$) and Reunion ($r^2 = 0.20$, Fig. 7), and at each study sites (FMR, $r^2 = 0.00$; TMR, $r^2 = 0.04$, SAL, $r^2 = 0.14$, SLE, $r^2 = 0.02$).

Table 5: Mean number and size of gamete per polyp at maturity in *Acropora austera*. The data are expressed per polyp. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

Region	Sites	Mean number of oocyte (sd)	Mean size of oocyte µm (sd)	Fecundity index
South Africa	All sites	9.60 (0.31)	592.65 (75.68)	57
	FMR	9.38 (1.96)	539.14 (19.33)	51
	TMR	9.83 (1.97)	646.16 (68.09)	64
Reunion	All sites	6.69 (0.28)	611.25 (62.28)	41
	SAL	6.49 (1.24)	655.29 (80.80)	43
	SLE	6.89 (1.85)	567.21 (31.74)	39

Table 6: Summary of Tukey HSD post-hoc test on fecundity estimates in *Acropora austera*. . Significance: * p<0.05, ** p<0.01, *** p<0.001, ns not significant. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

Variables	Study sites	FMR	TMR	SAL	SLE
Mean number of oocyte per polyp	FMR		ns	***	***
	TMR			***	***
	SAL				ns
Mean size of oocyte per polyp	FMR		***	***	ns
	TMR			ns	***
	SAL				***
Fecundity index	FMR		***	**	***
	TMR			***	***
	SAL				ns

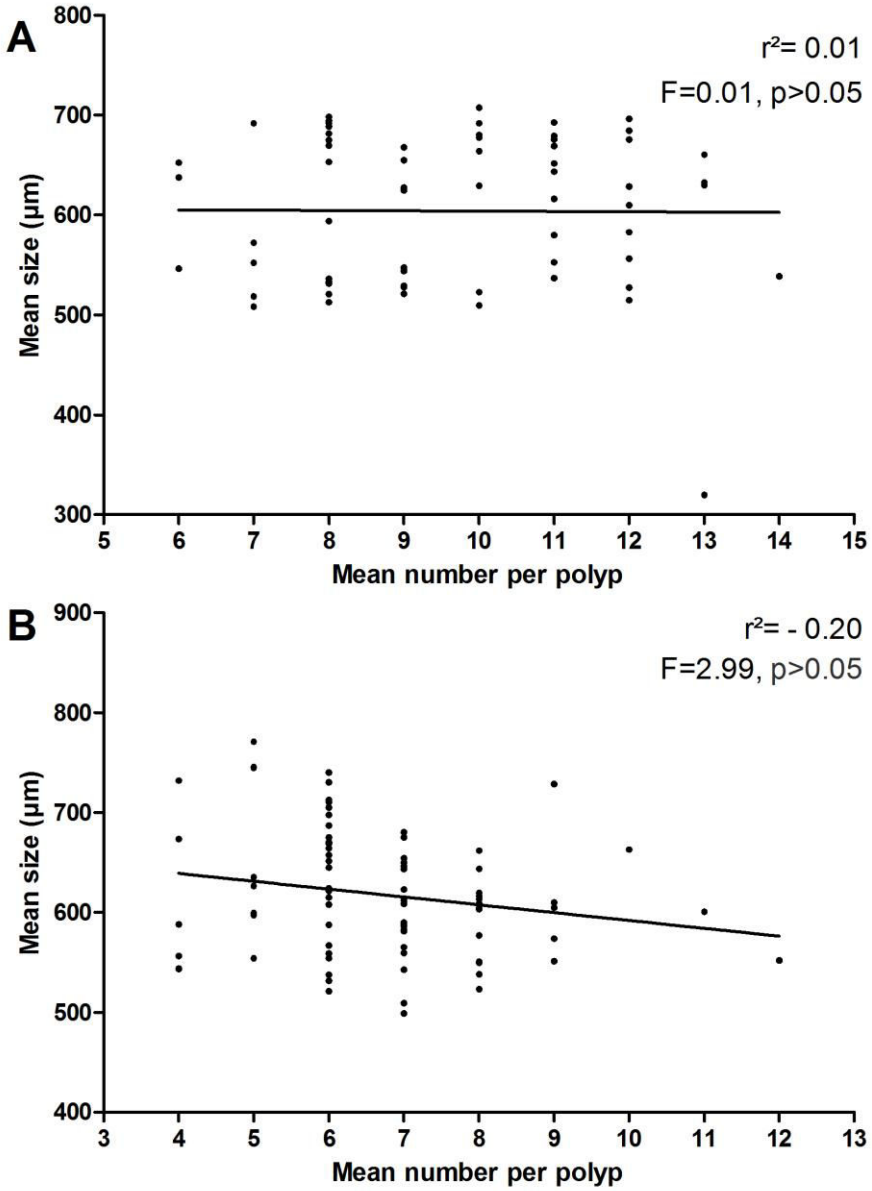

Figure 9: Relationship between the mean size and number of oocyte per polyp in *A. austera* in South Africa (A) and at Reunion (B).

5. Environmental influences

5.1.Seawater Temperature

Gamete maturation was initiated in *A. austera* during the rise in seawater temperature at the beginning of summer and ended during the summer maxima in the two regions (Fig. 10). The increase in gamete size was strongly correlated with the increase in seawater temperature in the two studied regions (r^2 = 0.79-0.92, Table 7). This correlation was significant for the oocyte at all study sites (r^2 = 0.64-0.91) and for the spermary at FMR (r^2 = 0.85) and SLE (r^2 = 0.88, Table 7).

The onset of gametogenesis occurred following the coolest months of the year in South Africa and Reunion. The start of gametogenesis (September to October) was, however, not directly link to the yearly temperature minima that occurred in August (22.82 ± 0.39°C, mean ± SD) in year 1 and July (21.78 ± 0.23°C) in year 2. In Reunion, August (23.71 ± 0.23°C) and September (23.67 ± 0.20°C) were the coolest months of year 1 and year 2 respectively but gametogenesis started between September and October in year 1 and between August and November in year 2. Similarly, spawning in *A. austera* did not always coincide with the summer peak in temperature. Spawning occurred one month after the summer peak (January, 27.04 ± 0.92°C) of temperature in year 1, but during the peak of temperature (February, 26.67 ± 0.37°C) in year 2 in South Africa. In Reunion, it probably took place one month before the summer maxima (March, 28.40 ± 0.25 °C) in year 1 and one month after it (February, 28.18 ± 0.44°C) in year 2 in Reunion.

5.2.Light intensity

A two-month delay was observed in the annual variation of light intensity between South Africa and Reunion (Figure 10). The peak in light intensity occurred between January and February in South Africa and between November and December in Reunion. In South Africa, the annual variation in light intensity followed a similar pattern as the variation in seawater temperature with one-month delay. A significant correlation was found between the increase in oocyte size and the seasonal changes in light intensity in South Africa ($r^2 = 0.82$) but this was not the case in Reunion ($r^2 = -0.30$, Table 7). The increase in spermary size was negatively correlated with light intensity in Reunion ($r^2 = -0.82$) but no significant was found in South Africa ($r^2 = 0.75$, Table 7). Spawning in South Africa coincided with the peak of light intensity in year 1 but occurred one month after the peak in year 2. In Reunion, no clear trend was observed in the timing of the spawning in *A. austera* and the annual variation in light intensity since it occurred three to four months after the summer maxima.

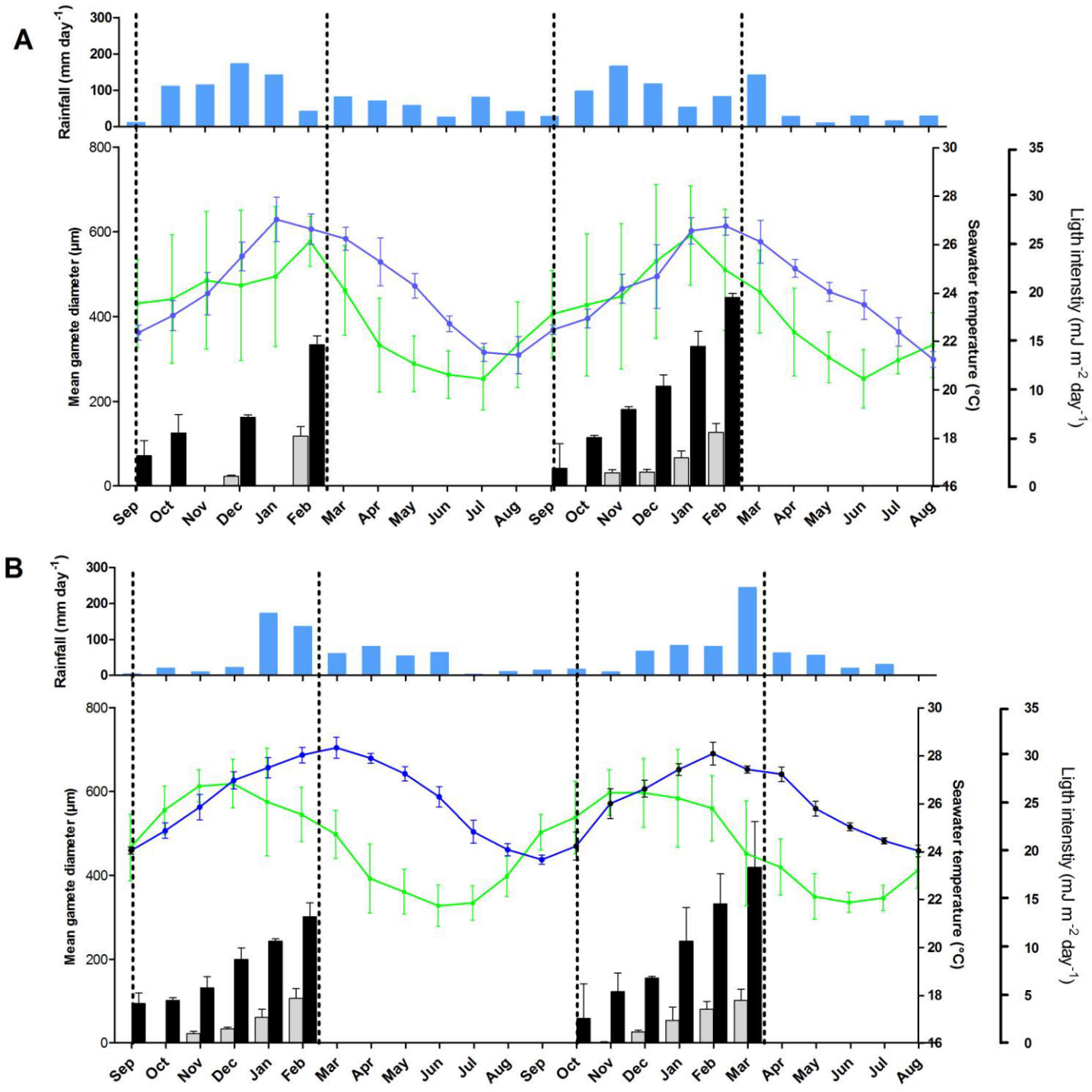

Figure 10: Changes in oocyte and spermary size relative to seawater temperature, light intensity and rainfall in *Acropora austera* on South African (A) and Reunion (B) reefs. The dashed lines indicate the breeding season, based upon the observed or inferred dates of spawning. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

Table 7: Summary of spearman product moment analysis between gamete size and three environmental factors in *Acropora austera* on South African and Reunion reefs. Significant correlations are in red (α = 0.05). S: size. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

		Gamete	Seawater temperature (T)	Light intensity (LI)	Rainfall (R)
South Africa	All sites	Oocyte	r^2 = 0.92 S = 67.86 × T - 1460.00	r^2 = 0.82 S = 37.38 × LI -590.40	r^2 = 0.02 S = 0.05 × LI + 199.76
		Spermary	r^2 = 0.82 S = 21.57 × T – 493.0	r^2 = 0.75 S = 11.33 × LI -195.10	r^2 = - 0.81 S = -0.54 × LI – 121.35
	FMR	Oocyte	r^2 = 0.91 S = 70.57× T - 1526.00	r^2 = 0.78 S = 34.66 × LI -519.40	r^2 = - 0.65 S = - 1.66 × R + 416.06
		Spermary	r^2 = 0.85 S = 22.08 × T - 662.40	r^2 = 0.58 S = 8.55 × LI -136.20	r^2 = - 0.84 S = - 0.55 × R + 117.26
	TMR	Oocyte	r^2 = 0.89 S = 62.72 × T - 1332.00	r^2 = 0.77 S = 32.88 × LI -489.50	r^2 = 0.05 S = 0.11 × R + 206.62
		Spermary	r^2 = 0.77 S = 39.67 × T - 947.50	r^2 = 0.45 S = 10.44 × LI -166.4	r^2 = - 0.73 S = - 0.75 × R + 151.13
Reunion	All sites	Oocyte	r^2 = 0.82 S = 58.59 × T - 1353.00	r^2 = - 0.30 S = - 13.75 × LI + 540.77	r^2 = 0.86 S = 1.22 × LI – 114.53
		Spermary	r^2 = 0.79 S = 33.17 × T - 850.20	r^2 = - 0.82 S = - 12.66 × LI + 375.91	r^2 = 0.79 S = 1.22 × LI – 114.53
	SAL	Oocyte	r^2 = 0.88 S = 84.92 × T - 2064.00	r^2 = - 0.58 S = - 31.91 × LI + 1021.60	r^2 = 0.70 S = 1.02 × R + 134.12
		Spermary	r^2 = 0.67 S = 38.69 × T - 1006.00	r^2 = - 0.82 S = - 11.75 × LI + 354.55	r^2 = 0.73 S = 0.33 × R + 25.57
	SLE	Oocyte	r^2 = 0.64 S = 46.15 × T - 1016.00	r^2 = - 0.43 S = - 20.09 × LI + 704.09	r^2 = 0.89 S = 0.63 × R – 59.51
		Spermary	r^2 = 0.88 S = 84.92 × T - 2064.00	r^2 = - 0.58 S = - 31.91 × LI + 1021.60	r^2 = 0.70 S = 1.02 × R + 134.12

5.3.Rainfall

In South Africa, the breeding season in *A. austera* occurred during the wettest season but no significant correlation was found in the annual variation of rainfall and the oocyte development (Table 7). The increase in spermary size was however negatively correlated with the increased in spermary site. In Reunion, gamete development was initiated during the dry season and spawning occurred following the peak of rainfall during the wet season. Significant correlations were observed between the annual variation of rainfall and the increase in oocyte and spermary size.

Result

Platygyra daedalea

1. Reproductive mode

A total of 370 colonies (185 colonies in South Africa and Reunion respectively) of *P. daedalea* were analysed over the two-year studied. *P. daedalea* was hermaphroditic in Reunion and South Africa, i.e. all polyps bear the male and female gametes at the end of gametogenesis. In contrast with *Acropora austera*, no male or female colony was observed during the two years of study. No developing embryos or planulae were observed in the histological samples over the two years of study proving that this coral was not a brooder but a broadcast spawner. This mode of reproduction was confirmed by the observation of gamete release in aquarium. The arrangement of gonad or morphology of gametes during development was similar between colonies collected in South Africa and Reunion. Each polyp contained 12 mesenteries filled with oocytes and spermaries intermingled within the mesogloea (Figs.11A-C). Two of these mesenteries were shared between polyps i.e. they were closely attached to each other. The spermaries were difficult to distinguish within the gonad as they developed as small rounded pile in between the oocytes (Fig. 11C). In some samples, the mature oocytes exhibited a dark hole on one side (Fig. 11D). Histology revealed that it was a cavity occurring around the stretched nucleus that had migrated toward the periphery of the oocyte. This cavity was observed in mature oocytes of colonies from South Africa and Reunion.

2. Gametogenesis

Four stages of oocyte development were identified in *P. daedalea*. During spermary development, only the late stages (Stage III and IV) were observed in the histological sections, probably because of their small size and their hidden position between the oocytes (Fig. 11C). The gamete development in *P. daedalea* is illustrated in Figures 12 and 13 and the mean size of each developmental stage is shown in Table 8.

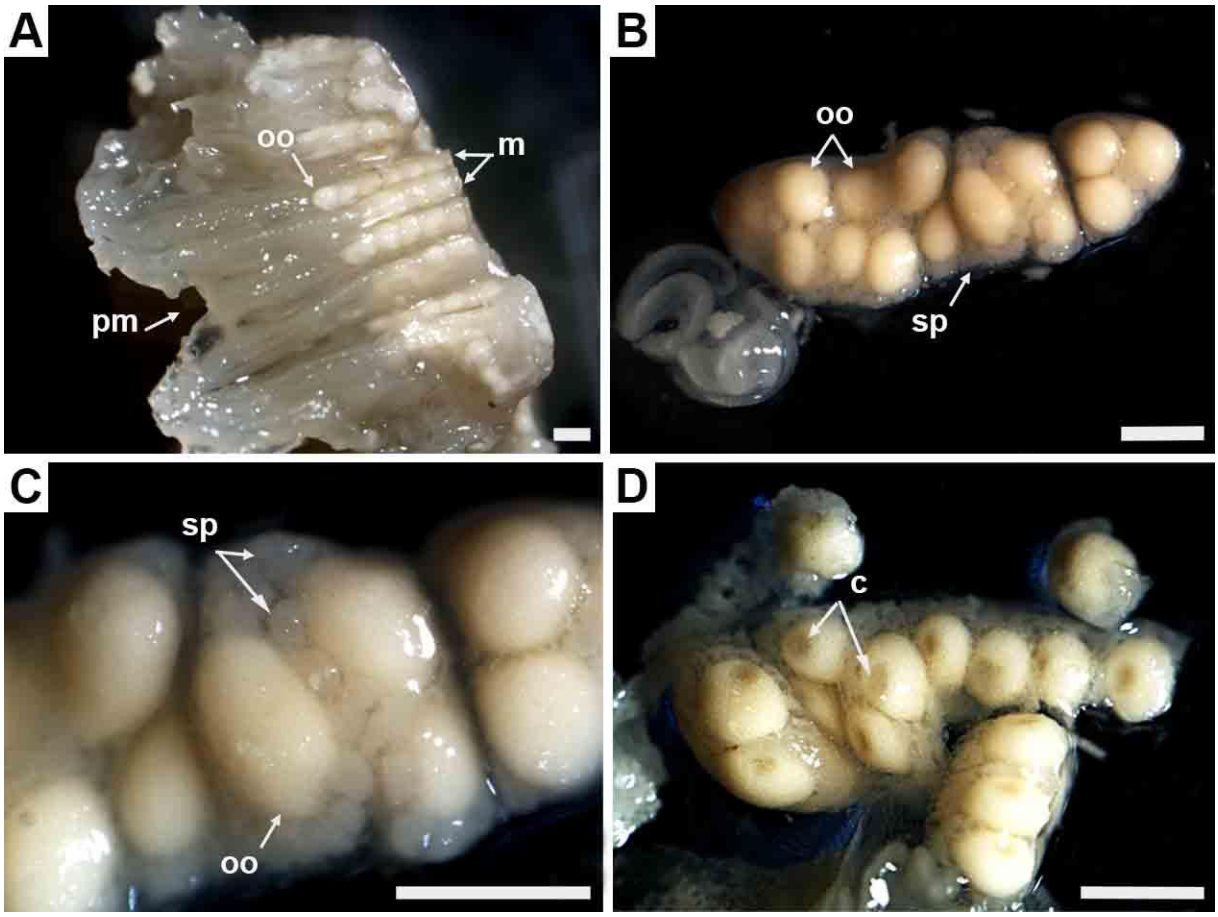

Figure 11: Dissection of *Platygra daedalea* polyps. A: Oocytes aligned in the polyp mesenteries. B-C: Dissected mesentery showing the oocytes and spermary. D: Mature oocytes with cavity in a dissected mesentery. c: cavity; m, mesentery; oo, oocyte; pm: polyp mouth; sp, spermary. Scale bars are 500µm.

2.1.Oogenesis

Stage I oocytes were irregularly shaped and ranged between 65-150 µm in histological section (Fig. 12A). Their cytoplasm was filled with numerous lipid vacuoles and their nucleus stained in bright pink. The nucleus occupied one third of the egg volume and this proportion was maintained from Stage I to Stage III. Stage II oocytes averaged 175 µm (Fig. 12B) and Stage III oocytes ranged between 200-250 µm in diameter (Fig. 12C). In mature Stage IV, the nucleus had migrated toward the periphery of the oocyte (Fig. 12D). It was stretched and adjacent to a cavity formed by the membrane of the oocyte. No zooxanthellae was present in the cytoplasm of Stage IV oocyte prior spawning.

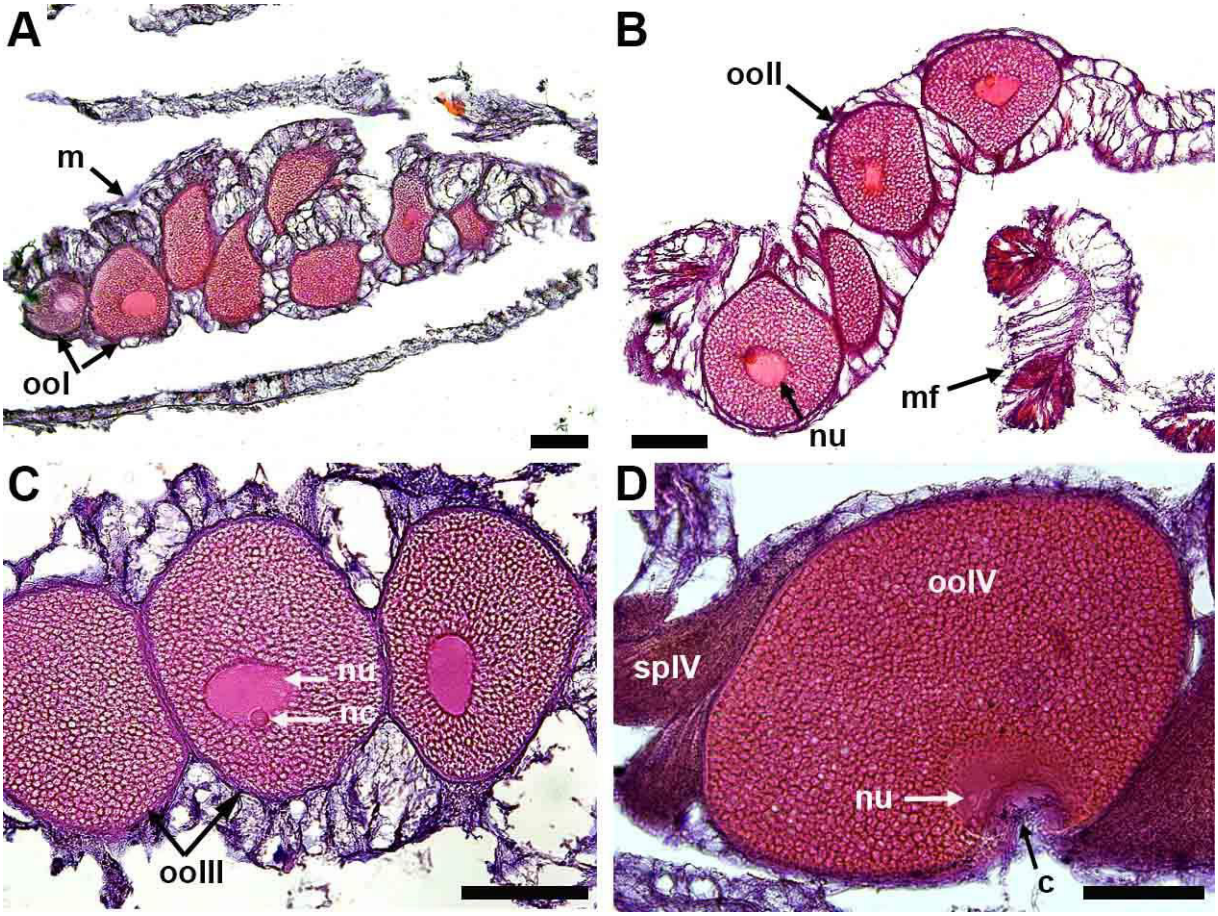

Figure 12: Oocyte maturation in *Platygyra daedalea*. A, Irregularly shaped Stage I oocytes in a mesentery. B, Stage II oocytes with bright pink nucleus. C, Stage III oocytes. D. Mature Stage IV oocyte prior spawning. c: cavity; m: mesentery; mf: mesenterial filament; nc, nucleolus; nu, nucleus; ooI, ooII, ooIII, ooIV: oocytes Stage I, II, III, IV respectively; spIV, spermary Stage IV. Scale bars are 100 µm.

Table 8: Mean oocyte and spermary sizes in histological preparations of *Platygyra daedalea* from Reunion and South Africa.

		Mean size (sd) µm		
		All sites	**South Africa**	**Reunion**
Oocyte	Stage I	121.29 (17.54)	126.15 (16.39)	117.91 (17.57)
	Stage II	169.38 (15.23)	174.71 (14.35)	168.88 (14.75)
	Stage III	227.58 (28.15)	233.28 (33.15)	224.31 (21.80)
	Stage IV	317.50 (46.40)	313.91 (42.62)	317.34 (48.82)
Spermary	Stage I	-	-	-
	Stage II	-	-	-
	Stage III	64.78 (12.45)	60.33 (8.85)	69.47 (17.52)
	Stage IV	138.20 (31.64)	142.68 (34.32)	135.87 (26.89)

2.2.Spermatogenesis

Stage III spermaries were 40-70 µm in histological sections and appeared as stretched ball between the Stage III oocytes (Figs. 13A and B). They stained dark pink to dark purple that allowed distinguishing them from the surrounding tissue and the oocytes; they remained nevertheless difficult to observe in most cases. Stage IV spermaries were 80-240 µm and characterised by strips that radiated from the centre toward the periphery of the gonad (Figs. 13C). At maturity, the spermaries were extremely dense, the distinctive characteristics of the spermatozoa were therefore not visible (Fig. 13 D).

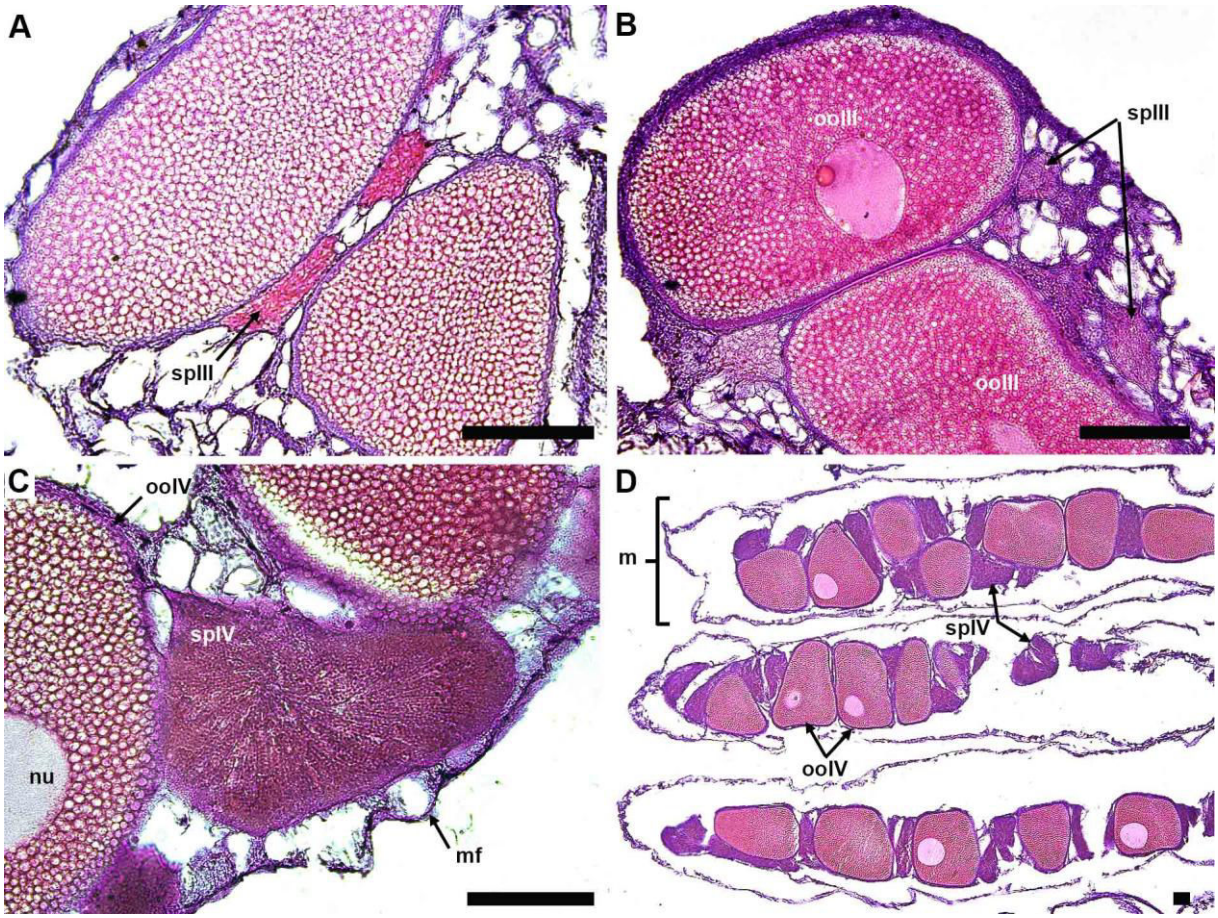

Figure 13: Spermary maturation in *Platygyra daedalea*. A-B, Dark stained Stage III spermaries constricted between Stage III oocytes one to two months prior spawning; C, closer view of a Stage IV spermary showing the strips radiating from the centre, D. Mature (Stage IV) spermaries and oocytes in the polyp mesenteries prior spawning. m: mesentery; mf: mesenterial filament; nu, nucleus; ooIII, ooIV: oocytes Stage III, IV respectively; spIII, IV, spermary Stage III and IV respectively. Scale bars are 100 µm.

3. Seasonality of gametogenesis

3.1.Seasonality of oogenesis

The seasonality in gamete development in *P. daedalea* is shown in Figures 14 and 15. *P. daedalea* showed an annual gametogenic cycle in South Africa and Reunion that last over 5-7 mo. It occurred at the same time of the year in the two regions despite small variations (± 1 month) between study sites and years. Oogenesis was initiated between September and October and spermatogenesis occurred 3-4 months later in January. Gametogenesis ended with the disappearance of mature gamete between February and April each year.

The oocyte development in *P. daedalea* off South Africa was highly synchronised between study sites and colonies. The peaks in the relative proportions of Stage I-IV occurred simultaneously at TMR and FMR. This trend was particularly visible during year 2 but may need further sampling to be verified in year 1. In Reunion, the oocyte development in *P. daedalea* was less synchronised between colony and study sites (Fig. 14), particularly at the beginning of the breeding season. The relative abundances of Stage I-III oocytes were not simultaneous between SLE and SAL. Nevertheless, the peak in Stage IV oocytes occurred in February in all colonies sampled off Reunion. Oogenesis may have started one month earlier in *P. daedalea* of Reunion than in South. Stage I oocytes were observed from September at SLE in Reunion while they appeared in October in South Africa. Overall, oogenesis seemed to be shorter in South Africa (five months) than in Reunion (six-seven months).

3.2.Seasonality of spermatogenesis

Spermaries became visible in January each year in *P. daedalea* of South Africa and Reunion and disappeared simultaneously with the oocyte between February and April (Fig. 15). The spermary development was less synchronised between polyps and study sites than the oocyte development. For example, several colonies lacked spermaries in January 2012 at FMR while, Stages III and IV spermaries were observed in the colonies collected at the same date at TMR. The same pattern was observed in Reunion during year 2 where colonies at SAL contained no spermary while the colonies at SLE had already Stage IV spermaries. Stages III and IV spermary were observed in the polyp mesenteries when the oocytes had reached the latest stage of development (Stages III and IV).

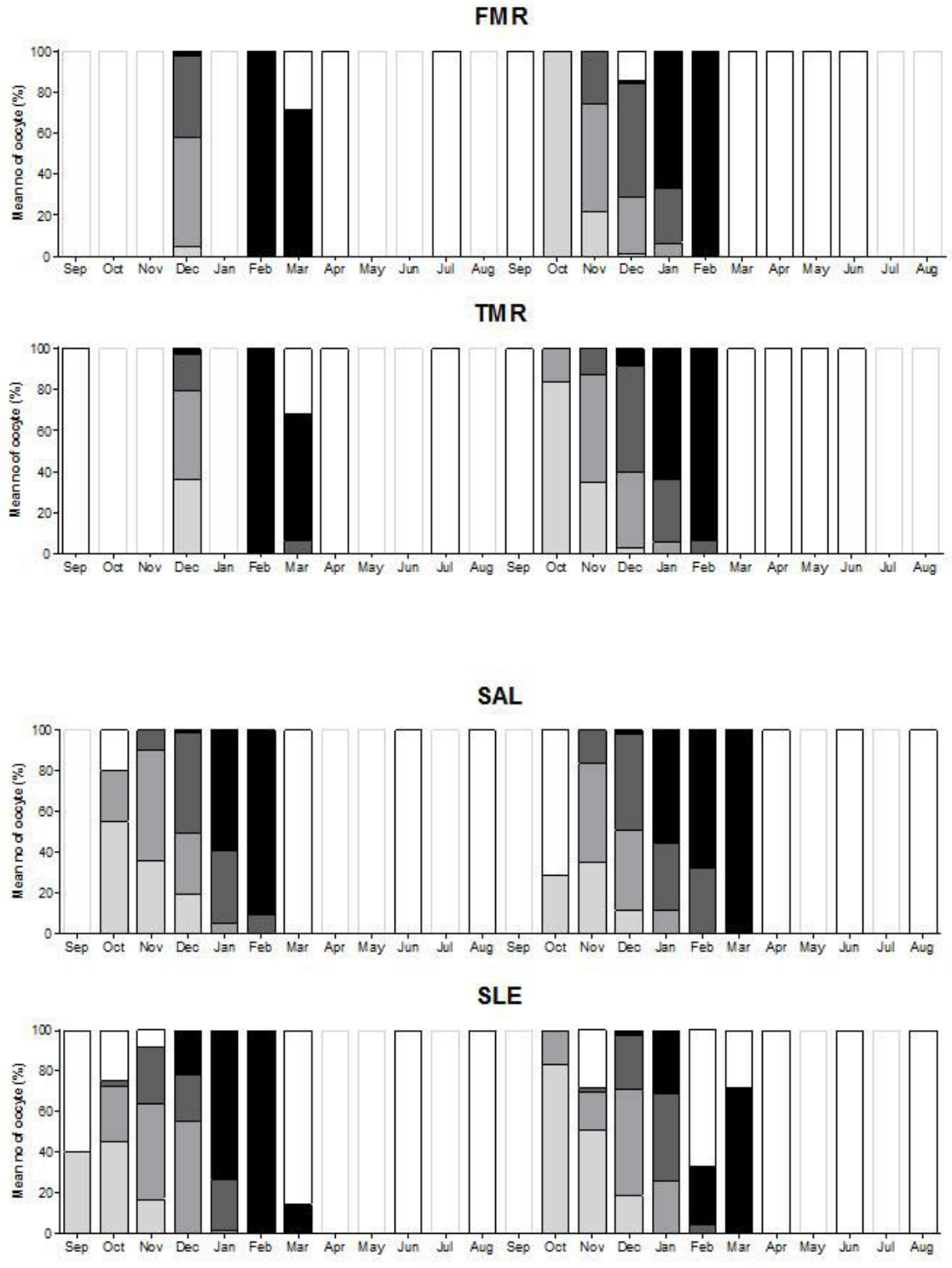

Figure 14: Seasonality in oocyte development in *Platygyra daedalea* at the study sites in South Africa (Two-mile reef, TMR, and Five-mile reef, FMR) and Reunion (la Saline, SAL, and St Leu, SLE). Light grey bars indicate no sampling (unclear).

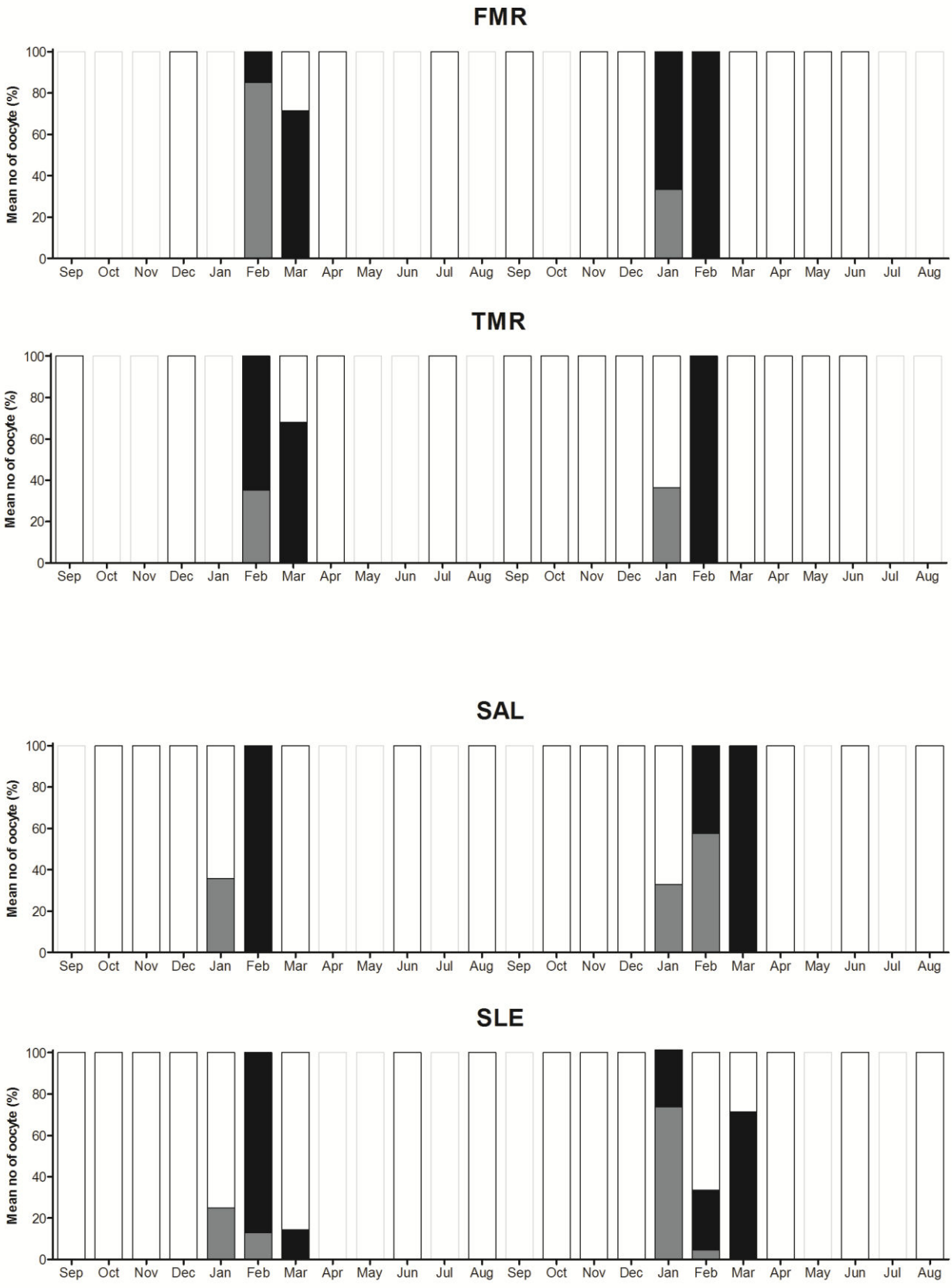

Figure 15: Seasonality in oocyte development in *Platygyra daedalea* at the study sites in South Africa (Two-mile reef, TMR, and Five-mile reef, FMR) and Reunion (la Saline, SAL, and St Leu, SLE). Light grey bars indicate no sampling (unclear).

4. Mean gamete size

The size of oocyte and spermary in *P. daedalea* increased regularly over time following the initiation of gametogenesis and did not reach a plateau close to maturity. The increase in oocyte size was best estimated by linear regressions (Goodness of fit, $r^2 = 0.81$-0.99), plotted per study sites and years (Fig. 16). It was however not possible to calculate the regression slope for the spermary size due to the paucity of data. The increase in oocyte size occurred at the same pace between the study sites of South Africa and Reunion as indicated by the lack of significant difference in the elevation of the regression slopes (F-test, $p>0.05$,

Table **9**). It was also similar between the two years of study at each study sites (F-test, $p>0.05$,

Table **9**

Table 4).

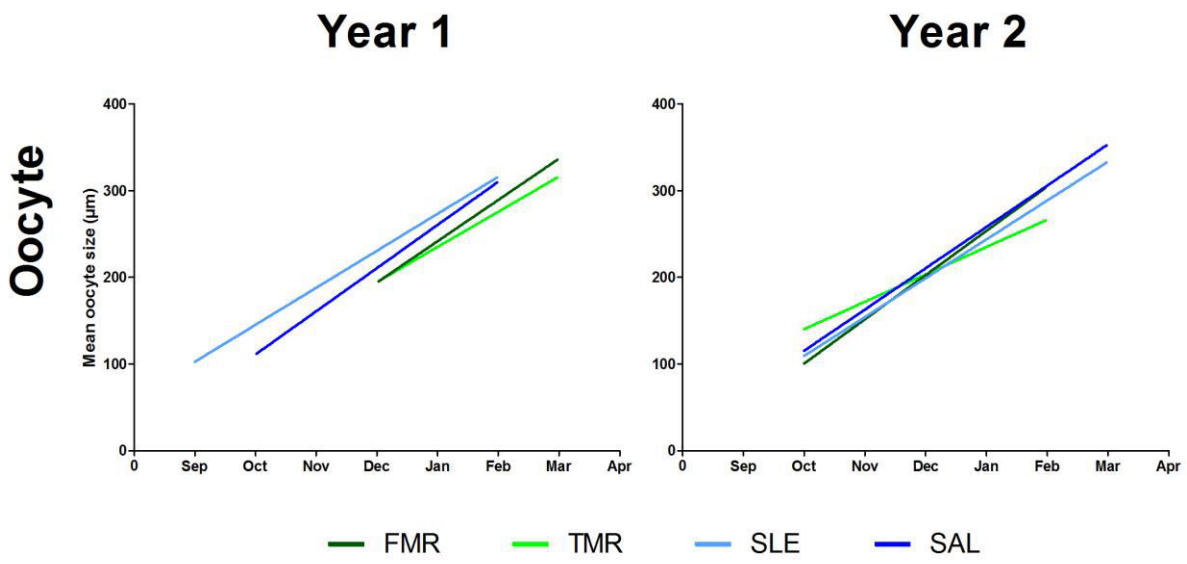

Figure 16: Linear regression of mean oocyte size in *P. daedalea* per study sites. Year 1: September 2010-August 2011; year 2: September 2011-August 2012. FMR: Five-mile reef, SLE: Saint Leu, SAL: la Saline, TMR: Two-mile reef.

Table 9: Summary of the F-test values for the comparison of the increase in oocyte size at each study sites. Year 1: September 2010-August 2011; year 2: September 2011-August 2012. Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns not significant.

Source of variation		F-test		
Fixed effect	**Random effect**	**F**	**df**	**p**
Between sites	Year 1	0.73	3	ns
	Year 2	0.39	3	ns
Between years	FMR	0.60	1	ns
	TMR	4.57	1	ns
	SAL	0.97	1	ns
	SLE	3.95	1	ns

5. Spawning

5.1.In situ observations of spawning

P. daedalea was not observed spawning *in situ* on the reef flat of Reunion despite the numerous night-dives (n=40).

5.2.Aquarium observations

Spawning in *P. daedalea* was observed in aquarium in South Africa but not in Reunion over three consecutive nights in 2012 (9-11th February), i.e. 2 to 4 days after full moon. The peak activity in spawning was observed at full moon +4 days when nine colonies over the ten collected spawn synchronously. Each night, sperm and egg bundles were released between 19:00 to 22:00, with peak release at 20:30. Individual polyps released single bundles in synchronous waves, every 15-30 minutes. Each bundle of gametes was visible in the polyp mouth for 0.5-2 h before release (Figs 17 A and B). During this time, they were rotated inside the polyp mouth, probably to compress the gametes and facilitate bundle expulsion (Fig. 17 C). After release, the polyp mouth continued to gape for several minutes (Fig. 17 D).

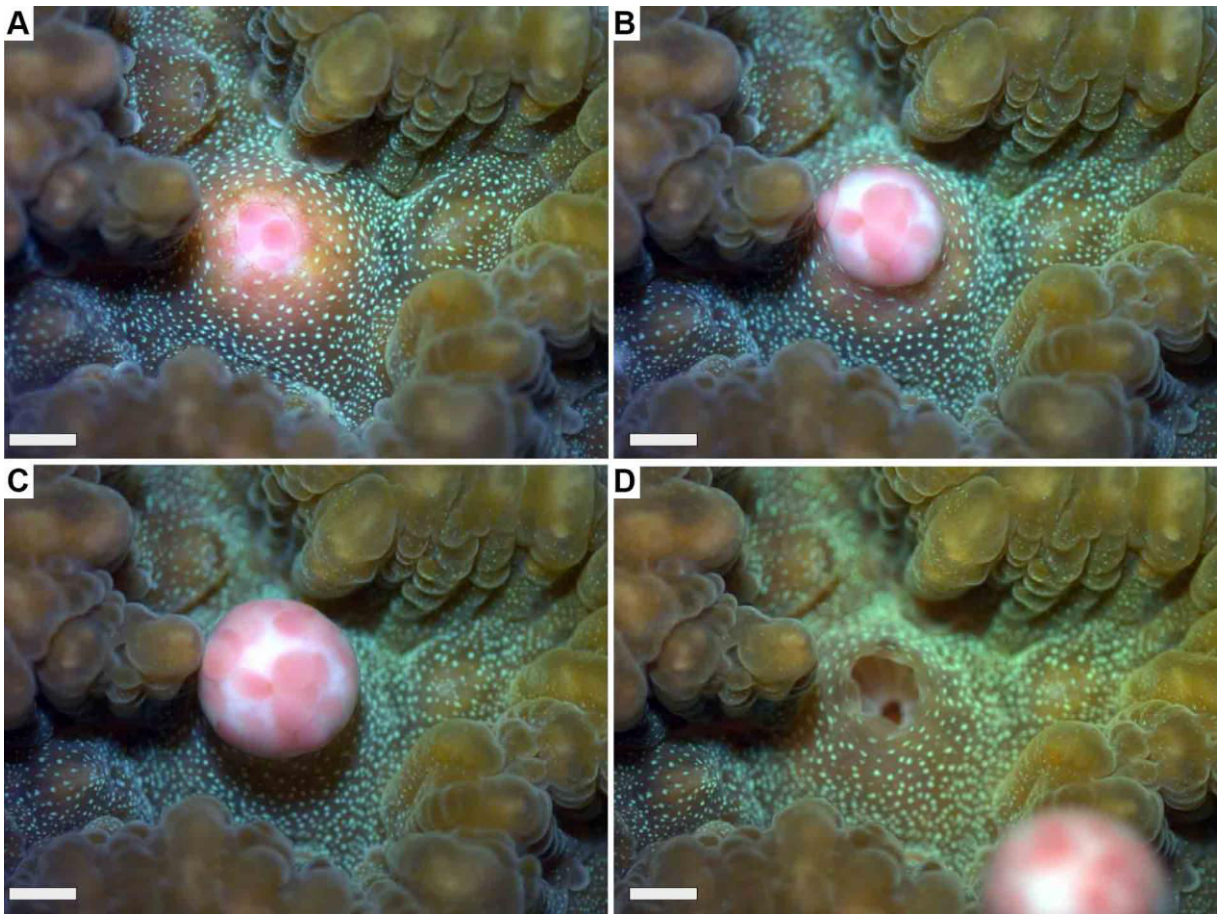

Figure 17 A-D: *P. daedalea* polyp at various stages of spawning. Scale bar: 1mm. Massé et al. (2013a)

5.3.Histology

The date of spawning in *P. daedalea* coincided with those of *A. austera* in Reunion and South Africa respectively, except in year 1 in South Africa where spawning in *P. daedalea* occurred one month later than in *A. austera*. In South Africa, a first decline in the number of colonies with mature gametes (Stage IV) was observed in *P. daedalea* on the 2^{sd} March 2011 since three colonies over the seven sampled were emptied. A limited spawning may have occurred

around this date that corresponds to the new moon period. An additional sampling was carried out on the 10th March, but this time, all sampled colonies except one were found with mature gametes. In Reunion, the disappearance of mature gamete in *P. daedalea* was synchronised between colonies at SAL. This was however less evident at SLE where one colonies of the seven sampled still contained mature gamete in March 2011. In 2012, a first decline in the number of colonies containing mature gametes was observed in February at SLE but not at SAL. No gamete was observed in the colonies collected in April 2012.

6. Fecundity

The mean number and oocyte size in mature samples of *P. daedalea* is given in Table 10. On average, the colonies of *P. daedalea* in South Africa contained twice as more oocytes than the colonies from Reunion and this difference in the mean number of oocyte was significant (ANOVA, $F = 286.35$, $p<0.001$). The average size of oocyte was significantly higher in Reunion than in South Africa (ANOVA, $F = 39.20$, $p<0.001$). However, this result was mainly due to the small oocyte size measured at TMR (Table 10), which was significantly lower than at the other study sites ($p<0.001$, Table 11). In contrast, there was no significant difference in the mean oocyte size of *P. daedalea* between FMR, SAL and SLE (Table 11). The fecundity index was significantly different between the two regions (ANOVA, $F = 255.15$, $p<0.001$) and appeared to be higher in South Africa than in Reunion (Table 10). TMR had the highest fecundity index and SLE the lowest.

Table 10: Mean number and size of gamete per polyp (± standard deviation) at maturity in *Platygyra daedalea*. The Fecundity index is calculated by multiplying the mean number and size of oocytes per polyp, divided by 100. The data are expressed per polyp.

Region	Site	Mean number of oocyte	Mean size of oocyte-µm	Fecundity index
South Africa	All sites	14,01 (3.20)	442,80 (32.89)	62
	FMR	13.26 (3.14)	456.63 (29.07)	61
	TMR	14.40 (3.20)	435.73 (32.76)	63
Reunion	All sites	6.14 (2.43)	460,56 (42.91)	28
	SAL	7.34 (2.24)	463.60 (39.40)	34
	SLE	5.01 (2.03)	457.68 (45.95)	23

Table 11: Summary of Tukey HSD post-hoc test on fecundity estimates in *Platygyra daedalea*. Significance: * p<0.05, ** p<0.01, *** p<0.001, ns not significant. FMR, TMR: Five-mile reef and two-mile reef respectively, South Africa. SAL, SLE: La Saline and St Leu, Reunion.

Variables	Study sites	FMR	TMR	SAL	SLE
Mean number of oocyte per polyp	FMR		ns	***	***
	TMR			***	***
	SAL				***
Mean size of oocyte per polyp	FMR		***	ns	ns
	TMR			***	***
	SAL				ns
Fecundity index	FMR		ns	***	***
	TMR			***	***
	SAL				***

A significant correlation was found between the mean size and number of mature oocyte per mesentery in *P. daedalea* off Reunion ($r^2 = -0.14$, $p<0.05$, Fig. 19) but this was not the case in South Africa ($p>0.05$). In Reunion, the correlation between these two parameters was negative meaning that the mesenteries contained either a few number of big oocytes or a high number of small oocytes.

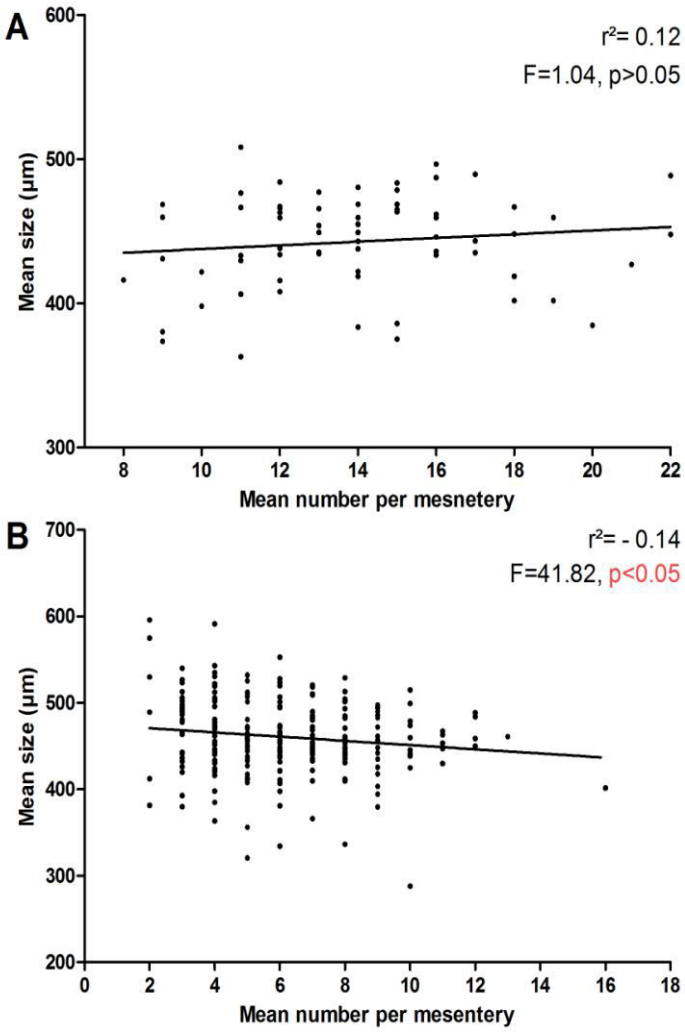

Figure 18: Relationship between the mean number and size of oocyte per mesentery in *P. daedalea* of South Africa (A) and Reunion (B).

7. Environmental influences

7.1. Seawater temperature

Like in *A. austera*, gamete development in *P. daedalea* was initiated during the rise in seawater temperature at the beginning of summer and spawning occurred during the warmest months of the year in the two study regions (Fig. 19). The increase in oocyte size in *P. daedalea* was strongly correlated with the increase in seawater temperature in South Africa and Reunion (r^2=0.90 and 0.87 respectively, **Table 12**: Table 12), except at SAL where the correlation was not significant. The r value for this correlation was nevertheless high and close to significance (r^2= 0.72). No significant correlation was found between the increase in spermary size and the seawater temperature in *P. daedalea*.

No clear trend between the onset of gametogenesis and the occurrence of the winter minima was observed in *P. daedalea.* Gametogenesis in *P. daedalea* started one to three months following the yearly lowest seawater temperature depending on year and study sites. Similarly, the timing of spawning did not always coincided with the peak of summer temperature. It occurred two months after the summer peak in temperature in year 1 but during the summer peak of temperature in year 2 in South Africa. In Reunion, spawning occurred one month before and one month after the peak of summer temperature in 2011 and 2012 respectively.

7.2. Light intensity

No significant correlation between the seasonal change in light intensity and the gamete development in *P. daedalea* was found in South Africa or Reunion except for the spermary development in Reunion (Table 12). The increase in spermary size was negatively correlated with the increase in light intensity at SAL and SLE. No clear pattern was noted between the onset of gametogenesis and the rise in light intensity as gametogenesis started one to three months following the minimum value of light intensity in South Africa and Reunion. Spawning however took place the month following the highest values of light intensity for the two year studied. This was not the case in Reunion, where spawning was two to three months after the peak of maximum light intensity.

7.3. Rainfall

No significant correlation between the annual variation in rainfall and the increase in gamete size in *P. daedalea* was observed in South Africa ($r^2 = 0.30$-0.69). The increase in rainfall was however significantly correlated with the increase in oocyte size in Reunion ($r^2 = 0.83$, Table 12). This was not the case for the spermary development. Spawning took place following the peak of rainfall in summer in Reunion. In South Africa, spawning occurred at the end of the wet season.

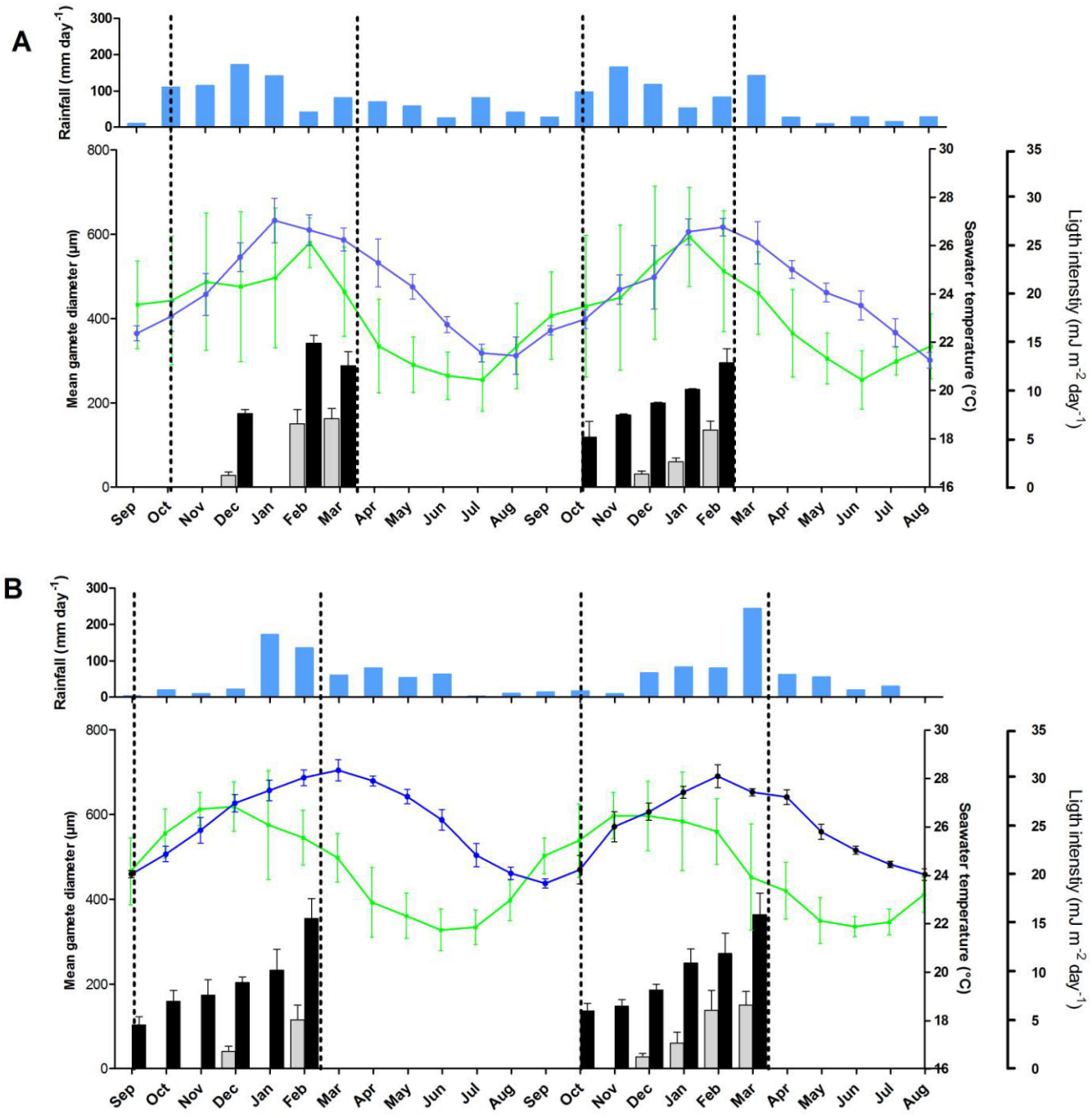

Figure 19: Changes in oocyte and spermary size relative to seawater temperature, light intensity and rainfall in *Platygyra daedalea* on South African (A) and Reunion (B) reefs. The dashed lines indicate the breeding season, based upon the observed or inferred dates of spawning. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef.

Table 12: Summary of spearman product moment analysis between gamete size and three environmental factors in *Platygyra daedalea* on South African and Reunion reefs. Significant correlations are in red (α = 0.05). S: size. FMR: Five-mile Reef, SAL: la Saline, SLE: Saint Leu, TMR: Two-mile Reef

		Gametes	Seawater temperature (T)	Light intensity (LI)	Rainfall (R)
South Africa	All sites	Oocyte	$r^2 = 0.90$ S = 53.07 × T - 1113.00	$r^2 = 0.62$ S = 19.08 × LI – 183.90	$r^2 = - 0.69$ S = - 1.17 × R + 355.78
		Spermary	$r^2 = 0.72$ S = 54.45 × T - 1325.00	$r^2 = - 0.07$ S = - 1.81 × LI + 136.20	$r^2 = - 0.65$ S = - 0.84 × R + 170.70
	FMR	Oocyte	$r^2 = 0.90$ S = 54.53 × T - 1158.00	$r^2 = 0.53$ S = 17.04 × LI – 147.10	$r^2 = 0.58$ S = 1.00 × R + 331.01
		Spermary	$r^2 = 0.69$ S = 51.25 × T - 1025.00	$r^2 = 0.20$ S = 4.65 × LI – 117.01	$r^2 = 0.62$ S = 1.45 × R + 127.60
	TMR	Oocyte	$r^2 = 0.80$ S = 40.17 × T - 796.60	$r^2 = 0.65$ S = 17.19 × LI – 154.00	$r^2 = 0.75$ S = - 1.08 × R + 334.32
		Spermary	$r^2 = 0.73$ S = 49.87 × T - 988.70	$r^2 = 0.08$ S = 2.88 × LI – 50.60	$r^2 = 0.30$ S = 0.55 × R + 264.70
Reunion	All sites	Oocyte	$r^2 = 0.87$ S = 50.83 × T - 1130.00	$r^2 = - 0.19$ S = - 7.15 × LI + 394.55	$r^2 = 0.83$ S = 0.96 × R + 149.51
		Spermary	$r^2 = 0.68$ S = 58.83 × T - 1532.00	$r^2 = - 0.83$ S = - 16.97 × LI + 507.86	$r^2 = 0.74$ S = 0.51 × R + 35.33
	SAL	Oocyte	$r^2 = 0.72$ S = 50.43 × T - 1124.00	$r^2 = - 0.70$ S = - 7.31 × LI + 395.76	$r^2 = 0.84$ S = 0.90 × R + 151.07
		Spermary	$r^2 = 0.70$ S = 41.44 × T - 1052.00	$r^2 = - 0.82$ S = - 16.58 × LI + 597.00	$r^2 = 0.71$ S = 1.24 × R + 178.00
	SLE	Oocyte	$r^2 = 0.82$ S = 44.04 × T - 953.20	$r^2 = - 0.21$ S = - 22.37 × LI + 771.73	$r^2 = 0.94$ S = 0.92 × R + 148.69
		Spermary	$r^2 = 0.67$ S = 51.37 × T - 1150.00	$r^2 = - 0.79$ S = - 5.22 × LI + 165.73	$r^2 = 0.69$ S = 0.89 × R + 135.40

Discussion

Sexual reproduction in *A. austera* and *P. daedalea* on the marginal and tropical reefs under study revealed similarities in their reproductive mode, gamete development and seasonality. There were, however, differences between the two regions in their fecundity and date of spawning (**Table 13**).

Table 13: Summary of the main results obtained in the present study and comparison between the two studied sites (South Africa and Reunion)

		South Africa	**Reunion**
A. austera	Reproductive mode, gonad arrangement, sex ratio	Broadcast spawner, protogynous hermaphrodite, pairs of short and long mesenteries	
	Seasonality in reproduction	September-February	(August) September-March
	Onset of spermatogenesis	October-December	November-December
	Length of gametogenesis	5-6 months	5-8 monts
	Gamete development	No difference	
	Reproductive output (fecundity index)	High	Low
	Size of mature gametes	No significant difference	
	Number of mature gametes per mesentery	High	Low
	Date of spawning	February	February-April
	Synchrony in gametogenesis and spawning	High	Low
P. daedalea	Reproductive mode, gonad arrangement, sex ratio	Broadcast spawner, protogynous hermaphroditic, 12 mesenteries per polyp including 2 shared ones	
	Seasonality in reproduction	October-March	
	Onset of spermatogenesis	January	
	Length of gametogenesis	5 monts	5-7 months
	Gamete development	No major difference	
	Reproductive output (fecundity index)	High	Low
	Size of mature gametes	Lower	Slightly higher
	Number of mature gametes per mesentery	High	Low
	Date of spawning	February or March	February or March
	Synchrony in gametogenesis and spawning	High	Low

1. Reproductive mode

A. austera and *P. daedalea* were hermaphroditic broadcast spawners in South Africa and Reunion with a single reproductive cycle per year. A similar reproductive regime was observed in *A. austera* (Harrison et al. 1984; Wallace 1985b; Babcock et al. 1986; Dai et al. 1992; Fukami et al. 2003; Carroll et al. 2006, Table 4) and *P. daedalea* (Dai et al. 1992; Miller & Mundy 2003; Mangubhai & Harrison 2008, Table 4) at other localities. Nevertheless, *P. daedalea* colonies have been reported to undergo two cycles of gametogenesis in equatorial regions (Oliver et al. 1988; Mangubhai & Harrison 2008). This phenomenon was not observed in the present study, despite intensive sampling conducted throughout the year. The two species *A. austera* and *P. daedalea* were protogynous hermaphrodites in South Africa and Reunion with oogenesis being initiated one to three months prior to spermatogenesis. This mode of reproduction has been reported in one acroporid in Florida (A. cervicornis, Vargas-Ángel et al. 2006) and was observed in *P. daedalea* off Kenya (Mangubhai 2007). In *A. austera*, the onset of spermatogenesis was positively correlated with the development of Stage III and IV oocytes (r^2=0.66and 0.52 respectively) and this trend was verified in South Africa and Reunion. A cavity was observed in mature oocytes of *P. daedalea* prior to spawning in dissected and histological samples of South Africa and Reunion. This cavity has not been reported in other studies on *P. daedalea*. It may favour the probability of the sperm to encounter the nucleus of the oocyte.

2. Gamete development

Gamete development was similar in both species in South Africa and Reunion and no significant difference was observed in the increase in gamete size during gametogenesis (F-tests, $p>0.05$). This result does not support the hypothesis that gamete development is impaired at higher latitude due to marginal environmental conditions (Wells 1957; Veron et al. 1974). In addition, the length of gametogenesis was expected to be longer in South Africa compared to Reunion, as it has been reported to extend with increasing latitude (Dai et al. 1992; Harii et al. 2001; Massé et al. 2013b). Gametogenesis in *A. austera* and *P. daedalea* was nevertheless similar between the two regions and occurred over a period of 5- 7 months in both species. Small variations in the length of gametogenesis were observed depending on the year or study site but they concerned a limited number of colonies. The length of gametogenesis in *A. austera* was slightly shorter than this reported in *A. cervicornis* of Florida (7- 8 months, Vargas-Ángel et al. 2006) and in *A. valida*, *A. hyacynthus* and *A. nobilis* on the Great Barrier Reef (9- 10 months, Wallace 1985b). This difference may however be due to

dissimilar sampling at the beginning and end of gametogenesis that may give a different estimation of the gametogenic length. No further comparative data on the length of gametogenesis in *A. austera* were found in the literature. Gametogenesis in *P. daedalea* in Reunion and South Africa (5-7 months) ranged within the value reported in *P. daedalea* of Kenya (Mangubhai & Harrison 2008) and Madang (Oliver et al. 1988), i.e. 6-7 months.

3. Fecundity

The fecundity index was higher in South Africa than in Reunion in *A. austera* (57 and 41 in South Africa and Reunion respectively) and in *P. daedalea* (62 and 28 in South Africa and Reunion respectively). *A. austera* off South Africa produced on average 43% more oocytes than its counterpart in Reunion but had a similar oocyte size (~600 µm). The oocyte size was similar to this reported in *A. austera* of Taiwan (Dai et al. 1992). Wallace (1999) suggested that variations in colony fecundity may emanate from the degree of crowding of the corallites. Nevertheless *A. austera* colonies were smaller and with shorter branches in South Africa than in Reunion, probably due to the intense surge (Riegl & Riegl 1996).

Colonies of *P. daedalea* contained more than twice the number of oocyte per mesentery in South Africa compared to Reunion. Their oocytes (442.80 ± 32.89 µm) were, however, smaller than in Reunion (460.56 ± 42.91 µm). A negative correlation between the size and number of oocytes was found in *P. daedalea* off Reunion, suggesting a 'trade-off' between the size and number of oocytes produced. Up to 60 oocytes per polyp were counted in a single polyp of *P. daedalea* in South Africa. This number is lower was however lower than this reported *P. daedalea* in Kenya (up to 100 oocytes per polyp), yet the oocyte size in *P. daedalea* was higher in South Africa than in Kenya (285 µm, Mangubhai 2007).

Scleractinian corals may divert resources away from sexual reproduction into other life functions in response to stress (Richmond 1987a; Gray 1989; Ward 1995; Vargas-Ángel et al. 2003). Egg production may therefore be indicative of the relative hostility of the environment (Price 1974; Williams 1975) and fecundity was expected to diminish in the subtropics compared to the low latitude reefs. The opposite pattern was observed in this study. In contrast with the first hypothesis, high fecundity is commonly considered an adaptation to ensure the maintenance of a population when the probability of survival is low (Price 1974; Williams 1975) and the stress is high (Grime 1977; Hall & Hughes 1996; Maltby 1999). Stressful environmental conditions at high-latitude may therefore increase the reproductive output of corals living in such environment. This idea seems to be supported by the fact that

sexual reproduction has been reported in corals in many marginal environments (Shlesinger & Loya 1985; Babcock et al. 1994; van Woesik 1995; Harii et al. 2001; Wilson & Harrison 2003; Fellegara et al. 2013). Furthermore, a greater oocyte size and number per polyp was found in *Acropora lutkeni* and *A. valida* on the high-latitude reefs of the Solitary Island (Wilson & Harrison 1997) compared to more tropical locations (21-25°N, Dai et al. 1992; Kenyon 1992). This pattern was also found in *Pocillopora damicornis* growing on the rocky reefs off South Africa (29°S, Massé et al. 2013b) compared to the tropical reefs of Panama (8°S, Glynn et al. 1991). The investment of energy into reproduction under stress conditions may be dependent on the level of stress (Gray 1989). Gray (1989) suggested that corals may invest more energy into reproduction at low levels of stress but fecundity would decrease with greater application of the stressor. Under the high-latitude conditions found in South Africa, the level of environmental stress may fall within the threshold tolerated by corals but be sufficient to enhance their reproduction and fecundity.

In addition, the difference in coral fecundity observed in this study may have been due to lower of level of disturbance on the South African reef compared to Reunion. The reefs in South Africa are in relatively good condition due to their remoteness and protection in marine reserves since 1986 (Schleyer et al. 2008a). In contrast, eutrophication and reef degradation due to coastal development has been reported in Reunion since the 1980's (Guillaume et al. 1983; Cuet et al. 1988; Montaggioni et al. 1989; Naim 2006; Tourrand et al. 2013). Eutrophication and in particular nutrient enrichment causes significant problems for coral reefs, such as enhanced growth of algae that compete with coral for light and a deterioration of water quality (Richmond 1993; Chazottes et al. 2002; Fabricius 2005; Hughes et al. 2007; Jessen et al. 2014). These effects are known to affect the coral health (Tomascik 1991; Richmond 1993; Fabricius 2005) and may have direct impact on its fecundity (Ward & Harrison 2000). For example, *Acropora longicyathus* and *A. aspera* exposed to elevated nitrogen produced significantly smaller and fewer eggs than in the control treatment (Ward & Harrison 2000). In Reunion, the high level of nitrates reported in some reef areas associated with submarine water discharge (Cuet et al. 1988; Chazottes et al. 2002; Tedetti et al. 2011) may have affected the fecundity of the two studied species.

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Table 14: Spawning months of *Acropora austera* and *Platgyra daedalea* in the southern hemisphere with corresponding lunar phases. * Observations made in aquaria

	Location	Coordinates	Month of spawning	Night(s) after full moon	Source
A. austera	French Polynesia	17°S	November	6	Carroll *et al*. (2006)
	Big Broadhurst Reef (GBR)	18°S	November	5-6	Harrison *et al*. (1984), Wallace (1985b), Babcock *et al.* (1986),
	Reunion	21°S	February-March	n/a	**Present study**
	South Africa	27°S	February-March	6-7*	**Present study**
P. daedalea	Kenya	3°S	February (major event)	0-14	Mangubhai and Harrison (2008)
	Kenya	3°S	August (minor event	0-14	Mangubhai and Harrison (2008)
	Orpheus Island (GBR)	18°S	November	5-6	Babcock *et al.* (1986)
	Magnetic Island (GBR)	19°S	November	4-7	Babcock *et al.* (1986)
	Magnetic Island (GBR)		October and November	6-7 (Oct), 1-8 (Nov)	(Willis et al. 1985)
	Bowden Reef (GBR)	19°S	November	5	Babcock *et al.* (1986)
	Reunion	21°S	February-March	n/a	**Present study**
	One Tree Island (GBR)	23°S	November	6	Miller and Mundy (2003)
	Heron Island	23°S	November	7	Nozawa and Harrison (2000)
	South Africa	27°S	February-March	2-4*	**Present study**

4. Seasonality and spawning

The breeding season in *A. austera* and *P. daedalea* occurred at the same time of the year, i.e. October to March in South Africa and Reunion. Small variations in the timing of the onset and end of gametogenesis were observed within the same species between study sites, a limited number of colonies differing in their onset by roughly a month. A similar period of gamete development (September to March) was observed in *P. daedalea* off Kenya (Mangubhai & Harrison 2008). No data was found in the literature on *A. austera* for comparison. These results contrast with those found in other studies at high latitude where gamete maturation and spawning were delayed relative to tropical reefs (Glynn et al. 1991; Harii et al. 2001; Wilson & Harrison 2003). Temperature is believed to control the time of the year at which corals breed and spawn gametes (Babcock et al. 1986) and late spawning at high latitude often coincides with a delay in the rise of sea temperatures compared to tropical reefs (Glynn et al. 1991; Harii et al. 2001; Wilson & Harrison 2003). In contrast, Babcock et al. (1994) found that spawning occurred in the same month on both temperate (Houtman Abrolhos) and tropical (Ningaloo) reefs in Western Australia, despite there being more than two months difference in timing of the seasonal temperature minima between the two regions. The seasonal variations in seawater temperature followed the same trend in South Africa and Reunion with one-month delay; the peaks in sea temperature were observed slightly earlier in South Africa (January to February) than in Reunion (February to March). These small differences in sea temperature fluctuations may explain the lack of delayed coral gamete maturation and spawning between these two regions.

The breeding season in the two studied species was strongly correlated with an increase in seawater temperature. Spawning occurred nevertheless either during, or after the summer peak in temperature, suggesting that other environmental factors may regulate the month of spawning. These factors may not be the same in South Africa and Reunion. In South Africa, the increase in light intensity was correlated with the increase in oocyte size in *A. austera* but not in *P. daedalea.* Nevertheless, spawning in the two species occurred following the summer peaks in light intensity over the two year of study. A combination of seawater temperature and light intensity may therefore control the timing of the breeding season and spawning in the subtropical reefs of South Africa. Solar radiation which is linked to light intensity has shown to control the timing of spawning in corals of Palau, Western Pacific (Penland et al. 2004) and in the Caribbean (van Woesik et al. 2006). In Palau, spawning in seven scleractinian species occurred following the rapid rise in light intensity in summer (Penland et

al. 2004). In the Caribbean, the solar insolation was correlated with the timing of the breeding season in 11 coral species but was not a good predictor of coral spawning (van Woesik et al. 2006). These studies suggest that corals may respond to an environmental cue in different ways depending on locality. In Reunion, no clear relationship was observed between the seasonal variations in light intensity and the timing of spawning or the increase in gamete size in the two studied species. The oocyte development in *A. austera* and *P. daedalea* was , however, correlated with the increase in rainfall and spawning took place following the months of heaviest rainfall over the two year studied. Few studies have investigated rainfall as a trigger for coral reproduction. Mendes & Woodley (2002b) showed that spawning in *Monstratea annularis* occurred before the heaviest month of rainfall. In addition a meta-analysis at 19 sites worldwide showed that coral spawning occurred either before or after the yearly peaks in rainfall (Mendes & Woodley 2002b). Heavy rainfall may lead to reproductive failure as it reduced the salinity at the water surface and affects fertilisation (Richmond 1996). It may also cause terrestrial runoff that has detrimental effect on the fertilisation rate and survival of coral larvae following fertilisation (see review in Fabricius 2005). In Reunion, large intrusion of fresh waters run-offs are observed on the reef during the rainy season (December to March, Cuet et al. 1988, Joint pers. com.) and this may serve as a trigger for coral spawning.

Table 15: Summary of the influence of selected environmental factors on gamete development in *A. austera* and *P. daedalea* off South Africa and Reunion.

			Environmental influences (correlation)		
		Month of spawning	**Seawater temperature**	**Light intensity**	**Rainfall**
A. austera	South Africa	February	Yes	Oocyte but not spermary development	Spermary but not oocyte
	Reunion	January-March	Yes	Spermary but not oocyte development	Yes
P. daedalea	South Africa	February-March	Oocyte but not spermary development	No correlation	No correlation
	Reunion	February-March	Oocyte but not spermary development	Spermary but not oocyte	Oocyte but not spermary

The months of sexual activity and spawning observed in *A. austera* off Reunion and South Africa did not corroborate previous observations made on these species in other localities (Table 14) and in other *Acropora* spp at Reunion (Vie Océane, Guillaume et al., unpublished data). Spawning in *A. austera* and *P. daedalea* was reported to occur in October and November with the rise in sea surface temperature on the Great Barrier reef and French Polynesia (Harrison et al. 1984; Wallace 1985b; Babcock et al. 1986; Carroll et al. 2006). In addition, spawning in other acroporids is regularly observed between October and November In Reunion (Vie Océane, Guillaume *et al*., unpublished data, Appendix 1). Nevertheless the spawning period noted in this study coincided with that reported in other reproduction studies in the Western Indian Ocean. Spawning occurred in February or March for *Pocillopora verrucosa* (Kruger & Schleyer 1998) and *Hydnophora exesa* (Hart pers com, pers obs) in South Africa (February to March), and in January to April for 19 species of *Acropora* and 3 species of Favidae, including *P. daedalea,* off Kenya (Mangubhai 2007). These observations suggest that they may be a clear change in the breeding seasonality between the Western and Eastern Indian Ocean. Further studies are required to verify if this trend in the breeding season is verified in other coral species and at other locality in the Western Indian Ocean.

Table 16: Spawning months of *Acropora austera* and *Platgyra daedalea* in the southern hemisphere with corresponding lunar phases.

	Location	Coordinates	Month of spawning	Night(s) after full moon	Source
A. austera	French Polynesia	17°S	November	6	Carroll *et al*. (2006)
	Big Broadhurst Reef (GBR)	18°S	November	5-6	Harrison *et al*. (1984), Wallace (1985b), Babcock *et al.* (1986),
	Reunion	21°S	Feb-Mar	n/a	**Present study**
	South Africa	27°S	Feb-Mar	6-7	**Present study**
P. daedalea	Kenya	3°S	February (major event)	0-14	Mangubhai and Harrison (2008)
	Kenya	3°S	August (minor event	0-14	Mangubhai and Harrison (2008)
	Orpheus Island (GBR)	18°S	November	5-6	Babcock *et al.* (1986)
	Magnetic Island (GBR)	19°S	November	4-7	Babcock *et al.* (1986)
	Magnetic Island (GBR)		October and November	6-7 (Oct), 1-8 (Nov)	(Willis et al. 1985)
	Bowden Reef (GBR)	19°S	November	5	Babcock *et al.* (1986)
	Reunion	21°S	Feb-Mar	n/a	**Present study**
	One Tree Island (GBR)	23°S	November	6	Miller and Mundy (2003)
	Heron Island (GBR)	23°S	November	7	Nozawa and Harrison (2000)
	South Africa	27°S	Feb-Mar	2-4	**Present study**

5. Synchrony in reproduction

Synchrony in gamete development and spawning was lower in Reunion than in South Africa. Early declines in the number of oocytes per polyp or mesentery were observed in *A. austera* and *P. daedalea* in Reunion over the two years of study (Figs 5 and 6). This may have been due to the sampling of non-fertile colonies or the early release of gametes prior the main spawning event. Split spawning, i.e. when coral divide spawning over two or more consecutive months, seemed to be common in *Acropora* spp and has been observed on several reefs such as Kenya (Mangubhai & Harrison 2006; Mangubhai 2007), the Great Barrier Reef (see for example Wallace 1985b; Willis et al. 1985; Hayashibara et al. 1993), French Polynesia (Carroll et al. 2006), Hawaii (Kenyon 1992), Japan (Shimoike et al. 1992; Hayashibara et al. 1993) and on the high-latitude reefs of the Solitary Islands, eastern Australia (Wilson & Harrison 2003). For example, only 84% of the gravid colonies of *A. austera* were observed spawning simultaneously in French Polynesia (Carroll et al. 2006). These observations suggest that split spawning in *A. austera* is not typical of Reunion and South Africa. It was also reported in *P. daedalea* on the Great Barrier Reef (Willis et al. 1985).

This result contrasts with the prediction of Baird et al. (2009b), which suggested that spawning synchrony peaks at mid-latitudes, and is lower near the equator and at high latitudes. Willis *et al.* (1985) suggested that split spawning may occur when the full moon period is early in the month following gamete maturity. However, the full moon did not differ between South Africa and Reunion and another explanation has to be found to explain this difference in spawning pattern. On the South African reefs, synchronous release of gametes may ensure high rates of fertilisation and genetic mixing (Harriott 1983b; Oliver et al. 1988; Harrison and Wallace 1990; Glynn et al. 1991), which may give rise to genetic combinations more adapted to a marginal environment. In Reunion, split-spawning may increase the chance of egg survival in the case of an unexpected events during spawning such as heavy rainfall, a tropical cyclone, or strong winds (that may drive the egg offshore), which are frequent during mid-summer (Richmond & Hunter 1990; Mendes & Woodley 2002b). Split spawning may also lead to less competition for space during spat settlement, which could favour recruitment success (Shlesinger & Loya 1985).

6. Conclusion

None of the hypotheses on coral reproduction at high latitude were verified in this study on two coral species studied. Active sexual reproduction was observed on the subtropical reefs of South Africa and Reunion, and the corals manifested similar gamete development and breeding season. In contrast, their fecundity and spawning synchrony were higher in South Africa compared to Reunion, suggesting that South African corals may allocate more energy to reproduction or that nutrient enrichment in Reunion affected coral reproduction. Overall, the similarities in the reproductive mode and seasonality in development observed in South Africa and Reunion suggest that the reproductive traits of the two species are similar in the south-western Indian Ocean. Babcock *et al*. (1994) proposed that corals at subtropical latitudes may exhibit a reproductive pattern inherited from parent colonies on tropical reefs (Babcock et al. 1994). Slight differences in the timing of spawning and the influence of environmental factors may, however, reflect local adaption in each region.

Appendixes

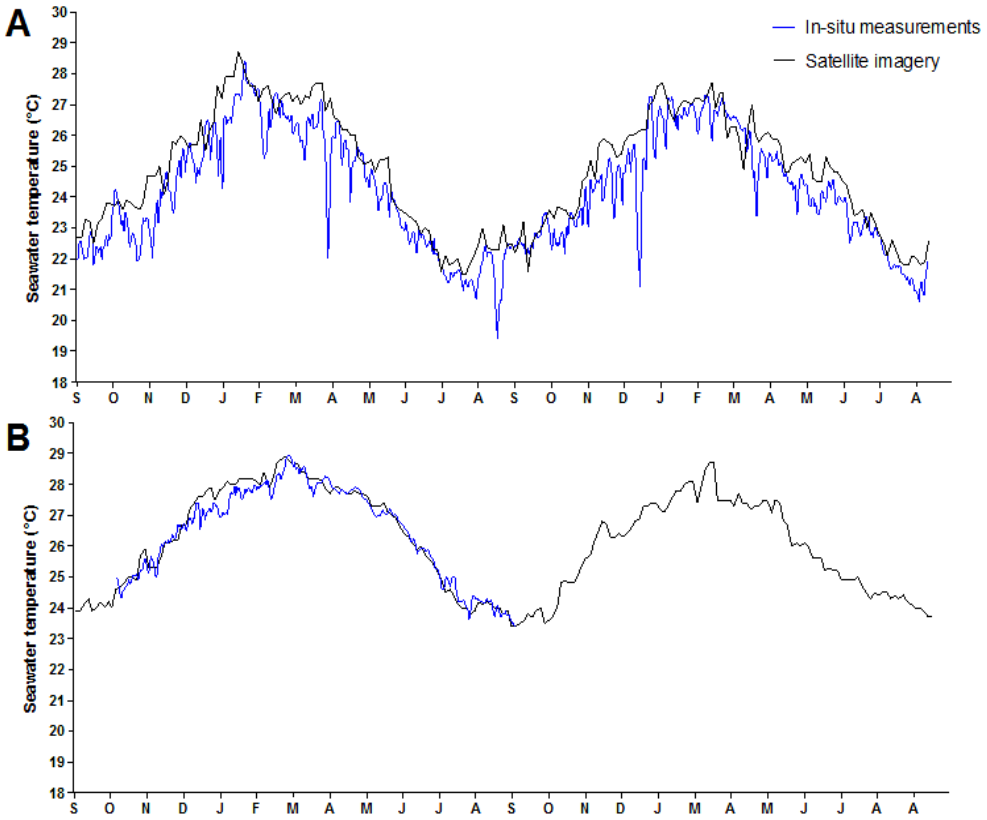
Appendix 1: Previous observations of coral spawning in Reunion. Source: M. Parmentier, M. Guillaume, Vie Océane, unpublished data. d, days; FM: Full Moon; ND: no data; NM: New Moon

Year	Month of spawning	Moon phase
1991	November	FM+1
1992	November	FM+3d
1993	November	FM+2-3d
1994	November	FM+2d
1995	ND	ND
1996	November	FM+1d
1997	ND	ND
1998	October	FM+1d
1999	September	FM+3d
2000	October	FM+4-5d
	November	FM+ 2-3d
2001	October	NM
2002	ND	ND
2003	September	NM-2-1d, NM
	October	NM
	November	FM+3-5d
2004	November	FM+3d
2005	October	FM+2-4d
2006	November	FM+2-4d
2007	September	FM+5d
	October	FM+1-2d
	December	FM+5
2008	ND	ND
2009	October	FM+5-6

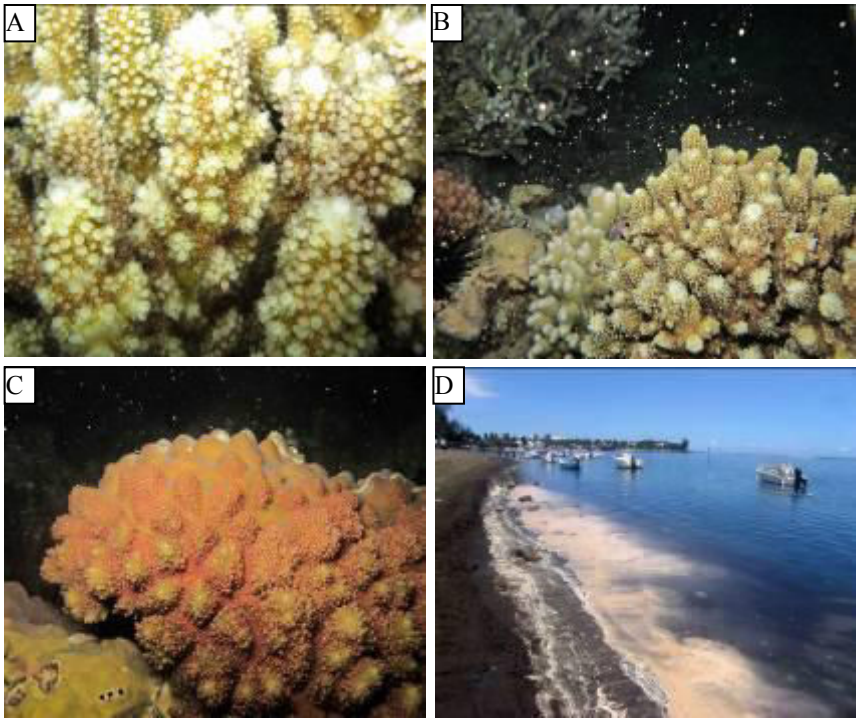
Appendix 2: Sampling dates of *A. austera* and *P. daedalea* colonies for monitoring of spawning in aquaria

	South Africa (TMR)	Reunion (SLE)
2011	• February (18/02)	• October (21/10) • November (19/11)
2012	• February (08/02)	• October (12/10) • November (10/11) • December (12/12)

Appendix 3: In-situ measurements (ORI, unpublished data) and satellite measurements (NOAA) of seawater temperature in South Africa (A) and Reunion (B)/



Appendix 4: Coral spawning in Reunion, November 2011.A and B: Spawning in *Acropora humilis* in the reef flat of St Leu. C: Colony of *Acropora sp* ready to release the egg/sperm bundles. D: Remains of coral spawning the following morning at Etang Salé.



Chapter 2:

A comparison of coral recruitment on a tropical (Reunion) and subtropical reef (South Africa) in the south Western Indian Ocean

Introduction

Recruitment, which is the process by which newly-formed individuals become part of the population (Sale 1990), is a key aspect for reef recovery and replenishment. In corals, this phase occurs following the development of the eggs into free swimming planula larvae that will settle on the reef and metamorphose into a polyp (Harrison & Wallace 1990). The rate of recruitment is dictated by complex interactions between adult fecundity, larval supply, connectivity between reefs, settlement success and limited early-mortality (Connell et al. 1997; Bellwood et al. 2004; Ritson-Williams et al. 2009). In addition, these processes may be affected by environmental changes or anthropogenic pressures such as coral bleaching (Hoegh-Guldberg 1999; Ward et al. 2000; Tamelander 2002) or water pollution (see review in Fabricius 2005) that can lead to the slow degradation of a reef (Richmond 1997; Hughes & Tanner 2000; Hughes et al. 2007).

Coral recruitment is reported to be lower on high-latitude reefs compared to tropical localities, putting these reefs at risk in the case of a major perturbation (Harriott & Banks 1995; Harriott & Simpson 1997; Hughes et al. 2002; Nakamura & Sakai 2010). This could be due to a combination of factors such as 1) decreased larval supply linked to the isolation of high-latitude reefs (Sammarco & Andrews 1988; Harriott 1992; Banks & Harriott 1996; Levin 2006; Nozawa et al. 2006), 2) marginal environmental conditions that may increase the early mortality of newly-settled corals (Wilson & Harrison 1997; Kleypas et al. 1999; Wilson & Harrison 2005) and 3) strong competition for space with temperate species (Harriott & Banks 1995; Banks & Harriott 1996; Holmes et al. 1997; Fairfull & Harriott 1999; Wilson & Harrison 2005). The interactions between these processes however remains poorly understood. There is nevertheless a growing need to understand the dynamics of coral recruitment on these marginal reefs as they may act as temperature refuges for tropical coral against global changes (Amat & Bates 2003; Riegl & Piller 2003; Lybolt et al. 2011).

In this study, coral recruitment was compared between South African (27-29°S) and Reunion Island (21°S) reefs to ascertain for differences along a latitudinal gradient. In particular, the 1) recruitment rate, 2) taxonomic composition and 3) position of recruitment were investigated on settlement tiles in a two-year study.

Materials and Method

1. Study sites and sampling

Coral recruitment was monitored over two years (October 2010 - October 2012) on recruitment tiles deployed at three study sites in South Africa and two study sites in Reunion (Fig. 1).

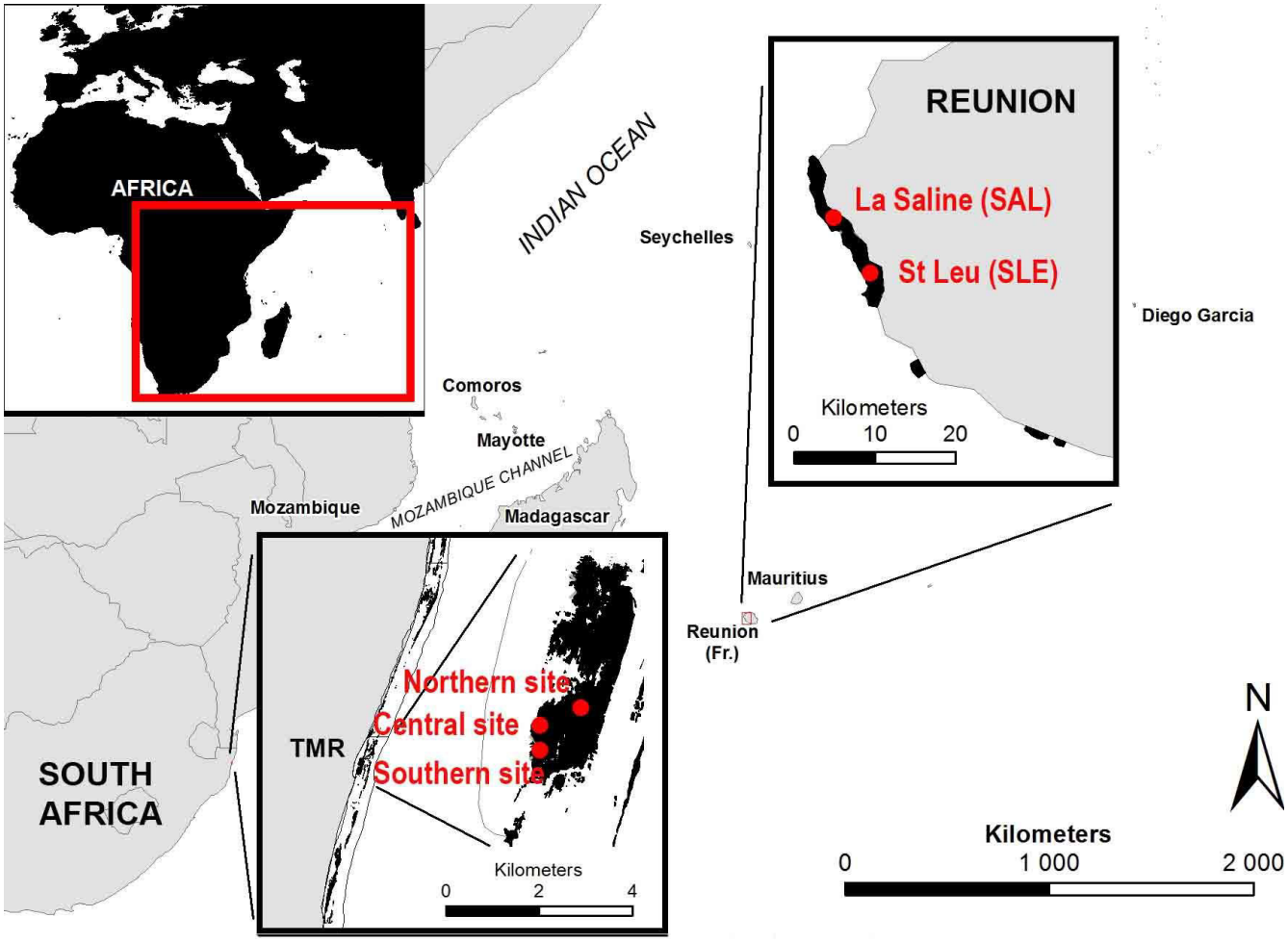


Figure 20: Location of the study sites for the recruitment surveys in South Africa and Reunion.

In South Africa, the reefs are characterised by high turbulence which precludes the use of regular forms of tile attachment to the reef (see Mundy 2000). The recruitment tiles were therefore attached to cement structures designed by Hart (2012, Fig. 2A). These structures were made to resist strong surge and persist over time for long-term surveys. Five tiles were attached to each arm of the Y-shaped concrete structures (Fig. 2A). In Reunion, the turbulence is not as strong as in South Africa and the Y-shape of the structures was too large to fit into the reef profile. Therefore, the arms were separated from each other and placed directly on the reef (Fig. 2B). Three Y-frame structures were located at each study site in South Africa and five of the nine arms were randomly selected for analysis. In Reunion, five arms were installed at each site. This sampling design corresponds to a total of 25 replicate tiles per study site in South Africa and Reunion.

Figure 21: Cement structure used for the fixation of settlement tiles in the coral recruitment survey in A) South Africa and B) Reunion. Y-frame

The recruitment tiles were replaced every six months by scuba-diving, simultaneously in the two regions. After bleaching for 2-4 days, they were dried in the sun and scanned under the microscope to search for coral recruits. Each recruit was measured and its position on the tile (upper surface or edge) was noted. The recruits were identified to the family level (Fig. 3) according to the descriptions provided by Babcock *et al*. (2003). The recruits that could not be identified because of damage or unknown morphology were categorised as "Other".

Figure 22: Skeletal prints of the most common coral families found on settlement tiles deployed on South African and Reunion reefs. a: Acroporidae; b: Pocilloporidae, c: Poritidae

2. Settlement tiles

A custom-designed tile with grooves (Fig. 4A, Hart *et al.,* submitted) was used as the main settlement substratum for coral recruitment in South Africa and Reunion. It aimed at providing a better substratum for settlement than the widely-used flat ceramic tiles (Fig. 4B), which are not representative of the natural substratum that has crevices and cracks. Grooved tiles were made of F16 fastcast polyurethane resin filled with calcareous sand to simulate the natural composition of the substratum. To allow for comparison between the two tile types, nine flat ceramic tiles were deployed simultaneously with grooved tiles in South Africa from April 2011 to October 2012 in the arrangement shown in (Fig. 5)**Figure 5**. The ceramic tiles were purchased from a supplier and cut to the same outer dimensions as grooved tiles (10 x 10 x 1 cm). Before the experiment, the flat and grooved tiles were soaked in saltwater for one to two weeks to allow leaching of chemicals.

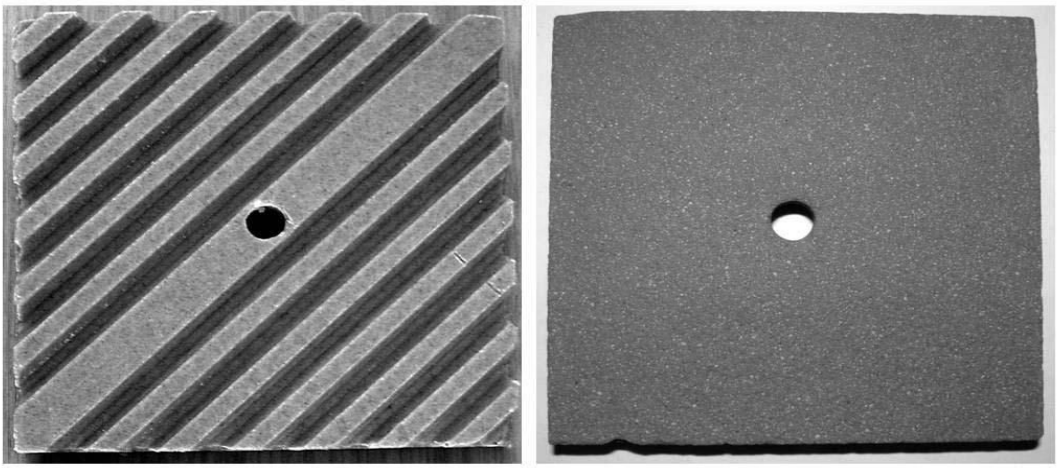


Figure 23: Grooved and flat settlement tiles before immersion

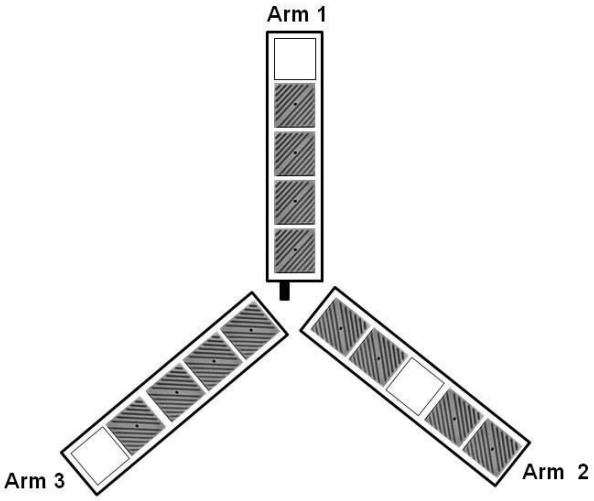


Figure 24: Y-frame structure used in the coral recruitment study off South Africa showing the position of the flat settlement tiles (white squares) on each arm and the position of the grooved resin tiles

3. Statistical analysis

3.1. Abundance of spat

The abundance of coral spat on tiles was found to be homogenous (Levene's test, $p < 0.001$), however log-transformation did not fully normalize the data. The residuals had nevertheless a normal distribution, hence, a general linear model (GLM) was used to analyse the data because it is fairly robust to small deviations from normality (Zar 1996). Nested ANOVA (Type III orthogonal) was used to test for interactions in recruitment rate between tiles, arms, and frames. The "frame" factor was considered only in the South African analyses as frames were not deployed in Reunion. "Year" and "season" were considered random factors while "arm", "frame", "site" and "regions" were the fixed factors. When a significant effect was detected, a Fisher LSD post-hoc test was used to locate significant differences between treatments.

3.2. Taxonomic composition and position of spat on tiles

Variations in the relative proportion of recruit family and position on tiles did not meet the assumptions of ANOVA. They were therefore analysed using the non-parametric Mann and Whitney U-tests. Since the sampling distribution of the U-statistic rapidly approaches the normal distribution in large samples (Siegel, 1956), this value was replaced by the z-adjusted statistic to accompany the respective *p*-value. All statistical tests were carried out using STATISTICA 10.0 (Stat Soft Inc. 2011).

3.3. Comparison of flat and grooved tiles

Due to the unbalanced and nested design of the data, Generalized Linear Mixed Models (GLMM, Venables & Dichmont 2004; Bolker et al. 2009) were used to assess the coral counts per tile, with the tile type and surfaces (edge or top) being considered as fixed effects and time and location as random effects. Each arm was nested within its concrete structure and site. In pilot analyses of the total spat counts using likelihood ratio tests, the negative binomial models performed consistently better than the Poisson model. The best results were achieved with a negative binomial error structure with parameterization of variance = $\mu (1 + \mu/k)$), where μ is the mean and k a constant; this formula was adopted throughout. Statistical modelling was performed with R statistical software, version 2.15.1 (R Development Core Team 2012), including the GLMM ADMB package, version 0.7.3 (Bolker et al. 2012).

Results

1. Comparison of grooved versus flat settlement tiles

The average number of coral spats (sd) on grooved and flat settlement tiles was 3.7 (3.5) and 3.6 (4.6) recruits per tile respectively. No significant difference was found in the mean number of recruits between the two tile types (GLMM, $p>0.05$). Grooved or flat surfaces had little influence on the taxonomic composition or seasonal variation of coral spat (Fig. 6), except in the Poritidae which were encountered in significantly higher number on grooved than flat tiles (GLMM, $p<0.05$). This family represented 3-5% of the total count, compared to Pocilloporidae and Acroporidae which accounted for 80 and 16% of the recruitment respectively. A marked difference was nevertheless observed in the position of coral spat between the two types of tile. Coral settled predominantly on the upper surface of grooved tiles (68% of the coral spat) but at the edges of the flat tile (57% of the coral spat) and these differences in spat position on the tiles were significant (GLMMs, upper surfaces, $p<0.001$; edges, $p<0.01$).

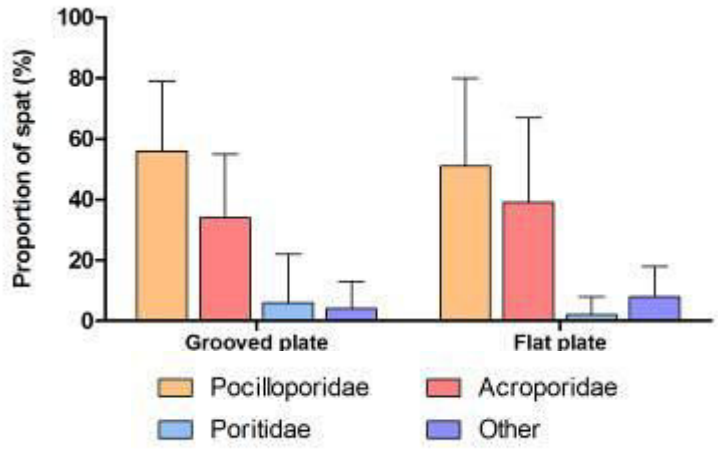


Figure 25: Coral composition on grooved and flat settlement plates deployed in South Africa.

2. Coral recruitment in South Africa and Reunion

2.1. Total count

The mean ± se annual recruitment rate was of 547.86 ± 35.40 recruits m^{-2} $year^{-1}$ in South Africa and 304.73 ± 27.21 recruits m^{-2} $year^{-1}$ in Reunion. Recruitment in South Africa was significantly higher than in Reunion (Nested ANOVA, $p<0.001$, Table 18**Erreur ! Source du**

renvoi introuvable.), and this trend was consistent between years, seasons and study sites (Nested ANOVAs, p<0.001, Table 18**Erreur ! Source du renvoi introuvable.**). The number of recruit per tile was highly variable and could range between 1-25 recruits per tile at the same study site (e.g. Southern site, summer of year 1). When all data were confounded, seasonal variation accounted for most of the variance (60%), followed by variation between tiles (23%) and regions (6%, Table 18). Despite the high variation observed at the tile level, no significant differences were detected in the total count of spats between tiles and arms in the global dataset (Nested ANOVAs, p>0.05, Table 18**Erreur ! Source du renvoi introuvable.**).

Table 17: Mean number (se) of coral recruits on tiles deployed over two years on reefs in South Africa and Reunion.

		South Africa				Reunion		
Year	**Season**	**All sites**	**North**	**Central**	**South**	**All sites**	**SAL**	**SLE**
1	**Summer**	10.17 (0.85)	15.11 (1.76)	8.63 (0.88)	7.60 (1.16)	4.84 (0.60)	5.20 (0.76)	4.37 (0.99)
	Winter	2.58 (0.30)	4.45 (0.57)	1.25 (0.26)	2.05 (0.37)	1.63 (0.21)	1.50 (0.27)	1.80 (1.03)
2	**Summer**	9.75 (0.80)	5.6 (0.68)	5.1 (0.53)	6.82 (0.80)	4.90 (0.63)	3.80 (0.73)	6.00 (0.96)
	Winter	4.51 (0.80)	4.17 (0.65)	0.92 (0.20)	2.12 (0.35)	1.04 (0.16)	1.12 (0.19)	0.96 (0.27)
TOTAL		5.48 (0.35)	9.68 (0.86)	3.82 (0.43)	4.29 (0.44)	3.05 (0.27)	2.99 (0.35)	3.12 (0.43)

2.2.Spatial and temporal pattern

Coral recruitment was highly seasonal in the two regions, the overall abundance of spat being approximately 4 times higher in summer than in winter (**Erreur ! Source du renvoi introuvable.**). The number of recruit per tile reached a maximum of 32 and 17 in the summer of year 1 in South Africa and Reunion respectively. Seasonal variation accounted for 55-60 % of the observed variability in Reunion and South Africa (Table 18). The difference in the total count of spat between the two years of the study was significant (Nested ANOVA, p<0.001), however this trend was not evident when considering South Africa and Reunion separately (Nested ANOVAs, p>0.05). Nevertheless, in South Africa, a higher proportion of recruit settled in 2011 than in 2012 (Nested ANOVAs, summer year 1/summer year 2, p<0.01; winter year 1/winter year 2, p<0.01). In Reunion, no difference was found between

the summers or the winters of year 1 and 2 (Nested ANOVA, summer year 1/summer year 2, p>0.05; winter year 1/winter year 2, p>0.05).

The recruitment rate was significantly different between the study sites in South Africa (Fisher LSD test, p<0.01) but not in Reunion. In South Africa, two to three times more recruits settled at the northern sites than the southern and central sites (**Erreur ! Source du renvoi introuvable.**). There was, however, no clear gradient in the diminution of recruitment rate from north to south in this region. In Reunion, SAL received more recruits than SLE during year 1 but the opposite trend was observed in year 2 (**Erreur ! Source du renvoi introuvable.**) and no difference in the total abundance of spat was detectable between the study sites and years (Nested ANOVAs, p>0.05, Table 18).

Table 18: Summary of nested ANOVA of the total count of recruits on settlement tiles in South Africa and Reunion over the two-year study. Tile (Arm): Factor "Tile" nested within the factor "Arm". Significance * P<0.05, ** P<0.01, *** P<0.001, ns not significant. Red text denotes significance

		df	F	p	Variance (%)
All data	**Region**	1	23,8418	***	6,53
	Site	2	11,5053	***	3,15
	Year	1	16,0970	***	4,41
	Season	1	220,3136	***	60,39
	Arm (Site)	4	1,1324	ns	1,86
	Tile (Site)	87	0,9918	ns	23,65
South Africa	**Site**	2	24,8532	***	28,63
	Year	1	2,8111	ns	1,62
	Season	2	114,6542	***	66,04
	Frame (Site)	4	0,6238	ns	1,44
	Arm (Frame)	6	1,3197	ns	2,28
	Tile (Arm)	64	1,2717	ns	46,87
Reunion	**Site**	1	0,00508	ns	0,00
	Year	1	0,40926	ns	0,38
	Season	1	58,60724	***	54,97
	Arm (Site)	8	2,41912	ns	9,08
	Tile (Arm)	44	0,86160	ns	35,56

2.3. Taxonomic composition of recruit

Pocilloporidae were the dominant spats on settlement tiles in both South Africa (62%) and Reunion (73%) (Fig. 7). Acroporidae were the second most abundant recruits in South Africa but were replaced in Reunion by the Poritidae. This difference in taxonomic composition between South Africa and Reunion was, however, not significant (Mann and Whitney U-test, $p<0.05$, Table 19), mainly because of the variation observed between years, seasons, and study sites. Unidentified or broken spat ("other") represented a varying proportion of spat ranging from 0-15%.

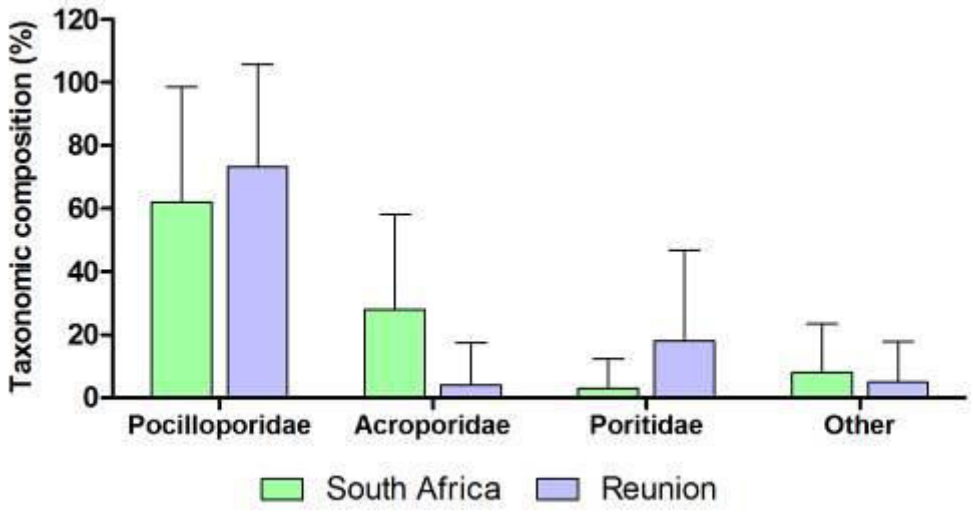


Figure 26: Taxonomic composition (mean and sd) of coral spat in South Africa and Reunion over the two years of study

Pocilloporidae were observed on settlement tiles throughout the year in South Africa and Reunion (Fig. 8), and their abundance did not differ significantly between seasons in the two regions (Mann and Whitney U-tests, $p>0.05$, Table 19). In contrast, Acroporidae were observed in summer only in South Africa and Reunion (Mann and Whitney U-tests, $p>0.05$). The seasonal variation in the abundance of Poritidae differed between South Africa and Reunion. They were observed in summer only in South Africa (Mann and Whitney U-test, $p<0.05$) but occurred in similar numbers in summer and winter in Reunion (Mann and Whitney U-test, $p<0.05$).

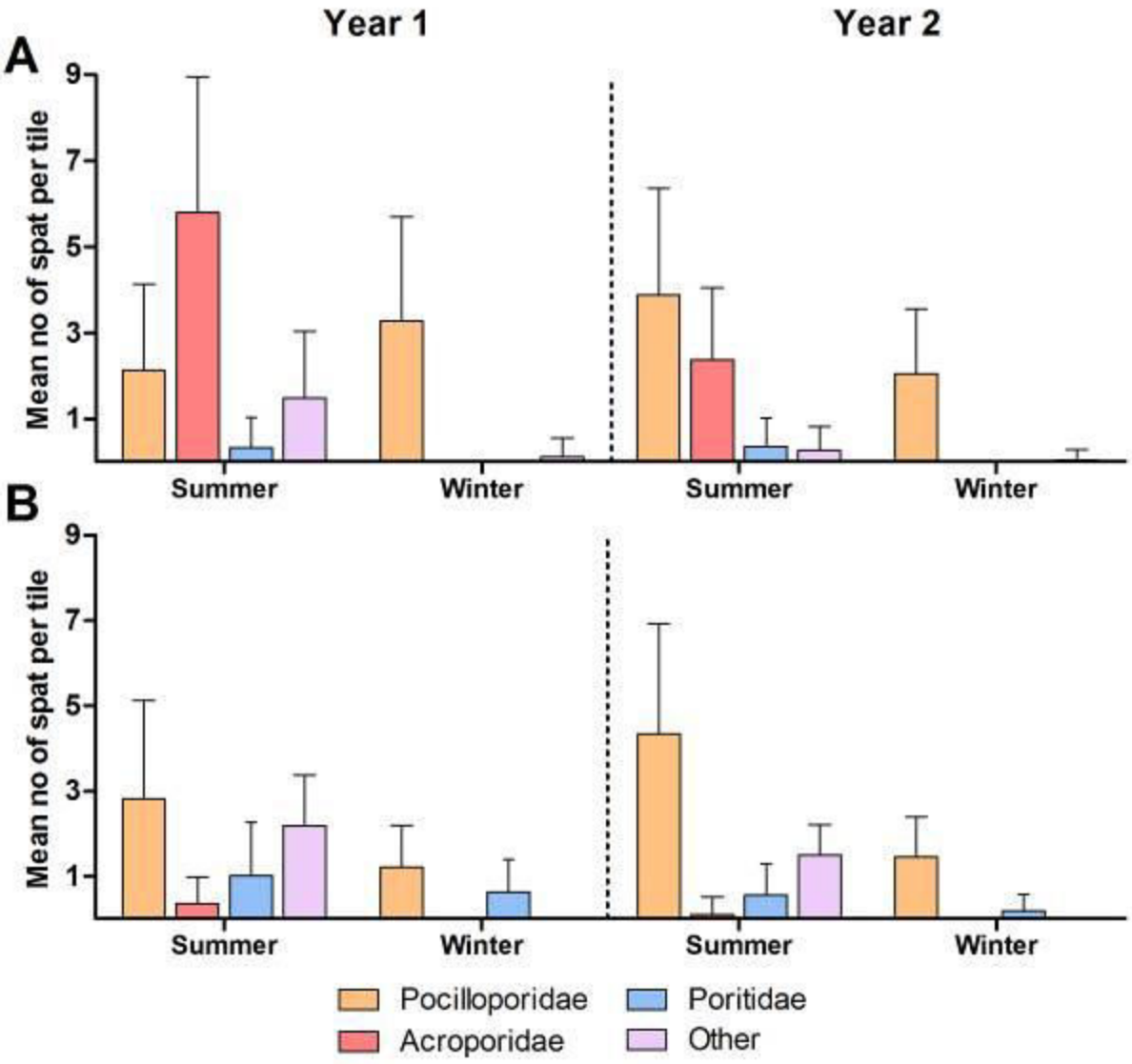


Figure 27: Seasonal and annual variation in the mean number (sd) of spat of the three main coral families per tile in South Africa (A) and Reunion (B)

A yearly variation in the abundance of spat from each coral family was observed in South Africa and Reunion. Pocilloporidae were more abundant in year 2 than year 1 in the two study regions but this trend was only significant in South Africa (Mann and Whitney U-test, $p<0.05$,

Table 19). Acroporidae were observed in higher numbers during year 1 than year 2 in South Africa and Reunion. This difference was, however, not significant (Mann and Whitney U-tests, $p>0.05$). The abundance of Poritidae was similar between years in South Africa and Reunion. It was slightly lower during year 2 compared to year 1 in Reunion but this trend was not significant (Mann and Whitney U-tests, $p>0.05$).

Table 19: Summary of Mann and Whitney U-tests comparing the taxonomic composition of coral spat between South Africa and Reunion. Only samples from summer (Oct-April) were considered for the comparison of Acroporidae and Pocilloporidae (random effect of season omitted) as they were absent or present in negligible numbers in winter. Red text denotes significance, * P<0.05, ** P<0.01, *** P<0.001. Vs: versus.

	Pocilloporidae			Acroporidae			Poritidae		
	Z-adjusted	p	Significance	Z-adjusted	p	Significance	Z-adjusted	p	Significance
South Africa vs Reunion									
All year	0.47	0.64	ns	0.26	1.00	ns	-0.02	0.98	ns
2011	0.51	0.61	ns	-0.53	0.59	ns	-0.83	0.40	ns
2012	1.69	0.09	ns	0.81	3.09	ns	-0.08	0.94	ns
South Africa									
Year 1 vs year 2	-2.73	0.01	*	1.16	0.25	ns	0.68	0.50	ns
Summer vs winter	-0.45	0.65	ns	-0.87	0.01	*	0.07	0.04	*
North vs Central	-0.72	0.47	ns	0.94	0.35	ns	-0.88	0.38	ns
North vs South	1.40	0.16	ns	0.23	0.82	ns	0.28	0.06	ns
Central vs South	0.00	1.00	ns	0.00	0.28	ns	-1.28	0.20	ns
Reunion									
Year 1 vs year 2	0.90	0.37	ns	-0.49	0.62	ns	-0.42	0.67	ns
Summer vs winter	0.95	0.34	ns	2.35	0.02	*	0.27	0.79	ns
SLE vs SAL	-2.17	0.03	ns	-0.60	0.55	ns	-0.09	0.93	ns

2.4.Position of recruits on tiles

The majority of coral spat settled on the upper surface of the settlement tiles compared to the edges. No significance difference was found in the position of recruits between South Africa and Reunion (Mann and Whitney U-test, $p>0.05$, Table 20). Similarly, no significant difference was found in the number of spat that settled inside ("in") and on the top of the grooves between the two study regions (Mann and Whitney U-test, "in", z adjusted -= 0.81, $p>0.05$; "top", z adjusted = -0.34, $p>0.05$, Fig. 9B).

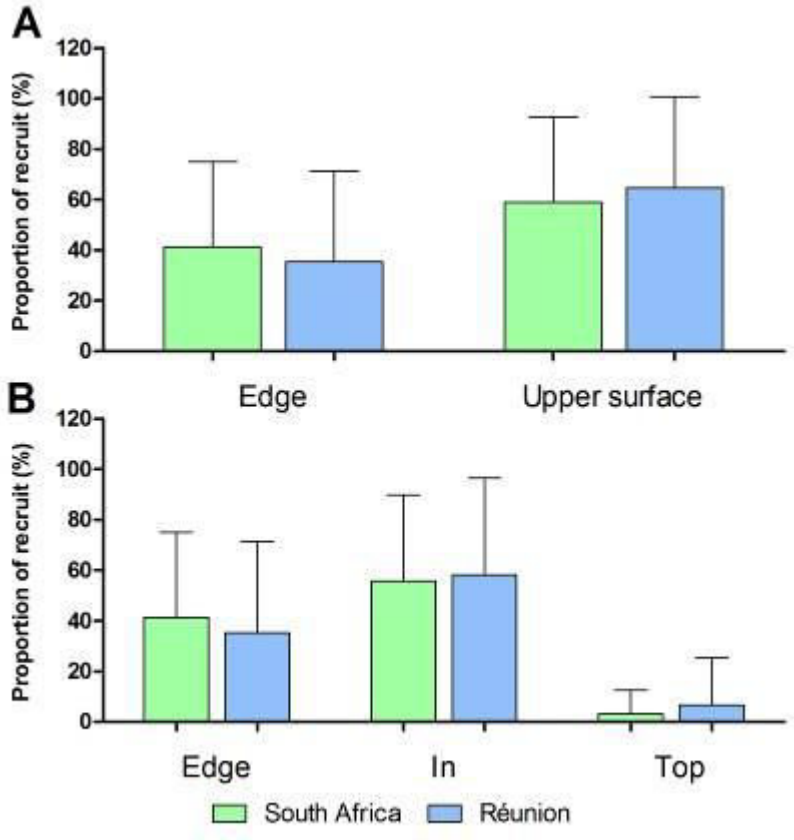


Figure 28: Spat position on settlement tiles in South Africa and Reunion. A: comparison between the edge and upper surface of the tiles. B: Further comparison including the position of spat in the grooves. Edge"Upper surface"= the top face of the flat tiles, "in" = in the grooves and "top" = on the top of grooves of grooved tiles.

Table 20: Summary of Mann and Whitney U-tests on coral spat position (edge versus upper surface) on settlement tiles between South Africa and Reunion. Redtext denotes significance, * P<0.05, ** P<0.01, *** P<0.001.

	Z-adjusted	p	Significance
South Africa vs Reunion			
All year	-0.297670	0.765955	ns
2011	-0.849967	0.395344	ns
2012	-0.085635	0.931757	ns
South Africa			
Year 1 vs year 2	0.932439	0.351110	ns
All site	-0.159128	0.873568	ns
North	1.224745	0.220672	ns
Central	0.974820	0.5950000	ns
South	-0,405840	0,684861	ns
North vs Central	-1.28535	0.198671	ns
North vs South	0.261911	0.793390	ns
Central vs South	0.421663	0.673271	ns
Reunion			
Year 1 vs year 2	-0.430013	0.667186	ns
Summer vs winter			
All site	-0.351335	0.725337	ns
SAL	-1,06662	0,286146	ns
SLE	-1,30943	0,190388	ns
SLE vs SAL	-0.358473	0.719989	ns

The pattern of spat location on the tiles was consistent between years, seasons and study sites (Fig. 11), and no significant differences were found in the relative proportion of spat settled on the edge or the upper surface of settlement tiles between South Africa and Reunion (Mann and Whitney U-tests, $p>0.05$, Table 20**Table 20**). Pocilloporidae settled in significantly higher number on the upper surface of the tile in South Africa and Reunion (Mann and Whitney U-tests, $p<0.05$, Table 21), while Poritidae were found in equal number on both surfaces (Fig. 11). A significantly higher number of Acroporidae was found on the upper surface of the settlement tiles in South Africa compared to Reunion (Mann and Whitney U-tests, $p<0.001$, Table 21).

Table 21: Summery of comparisons of spat position (edge versus upper surface) per coral family on settlement tiles between South Africa and Reunion. Red text denotes significance.

	Z-adjusted	p	Significance
Pocilloporidae			
South Africa	10,72158	0,000000	***
Reunion	2,796662	0,005164	**
Acroporidae			
Edge	0,548429	0,583398	ns
Upper surface	-8,09871	0,000000	***
Poritidae			
South Africa	1,844056	0,065176	ns
Reunion	0,151040	0,879945	ns

Discussion

1. Rate of recruitment

The recruitment rate was significantly higher in South Africa than in Reunion despite differences in environmental conditions between the two reef systems. An annual average of 548 recruits per m² was observed on the settlement tiles deployed for six months in South Africa compare to 305 recruits per m² in Reunion. The rate observed in South Africa ranged between those reported in the two previous surveys made at this locality (Glassom et al. 2006; Hart 2012, Table 6) and seemed therefore to be consistent between years. It was nevertheless higher than value reported at other subtropical locations (Harriott & Banks 1995; Nozawa et al. 2006) and on some tropical and equatorial reefs (see Table 22). The hypothesis that recruitment rate decreases with increasing latitudes (Harriott & Banks 1995; Harriott & Simpson 1997; Hughes et al. 2002; Nakamura & Sakai 2010) was therefore not verified in this study. Similarly, a decline in recruitment rate was not observed along the western coast of Australia as high spat densities, comparable with those reported in tropical studies on the Great Barrier Reef, was found at Lord Howe Island (31°S, Harriott 1992).

A comparison of settlement rates between different reefs is rendered difficult as studies use different tile materials, methods and timing of tile immersion (Harriott & Fisk 1987; Mundy 2000). In this study, the recruitment rate may have been influenced by the type of settlement tiles (grooved tile), the date of immersion (October and April), and the duration of deployment (6 months) of the settlement tiles. The simultaneous comparison of recruitment rates between widely used flat-ceramic tiles and the custom-designed grooved tiles yielded no significant difference in the overall abundance of spat. This suggests that tile type had little influence on the abundance of coral recruits. The settlement tiles were deployed in October and April each year in the study regions to cover the austral winter and summer. The date of immersion was based on the predicted spawning period in South Africa that occurs from December to February (Schleyer et al. 1997; Kruger & Schleyer 1998); the immersion of tiles in October allowed for natural preconditioning of the tiles prior to spawning. In Reunion, knowledge on the spawning date of corals is limited. During the two years of study; four acroporid species of the 21 species of *Acropora* found on the island (Faure et al. 2008), were observed spawning in October and November (Vie Océane unpublished data, pers obs). It is therefore possible that the deployment of the settlement tiles in October in Reunion may have been slightly late to catch the coral larvae from earlier spawning events. Nevertheless,

spawning in other coral species, including *Acropora* spp., was reported later in the season (December: *Porites lutea*, DENIS in press, February: *A. austera*, *P. daedalea* (Chapter 1) and *A. humilis* (pers. obs.)). Although October may have not been the best period to deploy settlement tiles in Reunion, the occurrence of multiple spawning events suggests that the settlement tiles were still able to collect much of the local coral recruitment. Finally, the period of tile immersion was chosen because of the technical constraints in comparing recruitment in two regions separated by 2500 km as well as the difficulty in accessing the South African reefs. A longer time or immersion may be a better sampling approach if settlement is protracted, nevertheless it may increase the probability of post-settlement mortality and overgrowth by other benthic organisms that makes the recruits indiscernible on tiles (Glassom et al. 2006). Mortality may therefore offset new settlement on tile deployed for longer periods (Glassom et al. 2004; Glassom et al. 2006). In the literature, the length of tile immersion varies from one (see for example Soong et al. 2003) to 16 months (see for example Glassom et al. 2006) and does not explain alone the variations observed in recruitment rates between reefs.

Recruitment at high latitude is believed to be reduced due to reef isolation that may limit the larval supply from other reefs following spawning events (Harriott 1992; Harriott & Banks 1995; Banks & Harriott 1996; Nozawa et al. 2006). However, the oceanographic systems on the South African reefs may limit their isolation. The reefs are washed by the Agulhas Current that originates from the Mozambique Channel (Saetre & da Silva 1984), and the East Madagascar Current (Lutjeharms 1981). This current transports warm waters of the Indian Ocean along the narrow continental shelf toward southern latitudes at a maximum velocity of 1.5 m s^{-1} (Schumann 1988; Lutjeharms 2006). It was predicted to transport coral larvae with it as these may remain in the water from days to several months before settlement (Richmond 1987b; Wilson & Harrison 1998; Nozawa & Harrison 2000). Recent genetic studies have, however, shown discontinuity between the coral populations of South Africa and Mozambique, suggesting that this scenario is unlikely (Ridgway et al. 2001; Macdonald et al. 2011; Montoya-Maya 2013). It will be therefore interesting to carry out genetic studies on newly-settled corals to investigate the origin of the larval input on the South African reefs.

An alternative hypothesis to explain the high rate of recruitment in South Africa may be the retention of larvae on natal reefs due to surface currents at the time of spawning (Morris 2009; Montoya-Maya 2013). Calm conditions and/or the alternation of opposite winds that drive the

surface currents at this period (Morris 2009) may promote the entrapment of larvae and their retention on natal reefs (Montoya-Maya 2013). Such limited dispersal may maintain the larvae close to a substratum suitable for settlement and limit their loss both downstream and offshore. Active sexual reproduction (see chapter 1, Schleyer et al. 1997; Kruger & Schleyer 1998) and high coral fecundity have been reported on the South African reefs (Chapter 1, Kruger & Schleyer 1998). A high level of local larval production and retention on the reefs may have contributed to the high rate of settlement observed at this latitude.

In Reunion, the recruitment rate measured on the reefs was close to that reported in French Polynesia (17°S, Gleason 1996) or Taiwan (24°S, Soong et al. 2003) but was much lower than at other tropical locations such as Japan (23°S, Nakamura & Sakai 2010) or the Great Barrier Reef (15-16°S, see for example Wallace 1985a; Fisk & Harriott 1990). Two hypotheses may explain the low rate of recruitment in Reunion. First, the larval supply from other reefs may be limited in Reunion, because of its relative isolation in the Indian Ocean. The island is washed by the South Equatorial Current (SEC) that crosses the Indian Ocean for 6500 km before reaching the Mascarene Plateau (Schott et al. 2009). The modelling of the oceanographic currents around Reunion has shown there may be some degree of unidirectional larval transport from the nearby island of Mauritius under particular environmental conditions such as storms and cyclones (Crochelet et al. 2013). This input may, however, be negligible because Mauritian reefs are small size. The model developed by Crochelet and co-authors (2013) furthermore predicts that larvae spawned in Reunion may be carried away from the island to southern latitudes, thereby limiting self-recruitment on the reefs. Secondly, fecundity in the two coral species under study was lower in Reunion than South Africa (Chapter 1). The larval supply from local coral colonies may therefore be limited and may be one of the explanations for the low recruitment rate observed in Reunion.

Low recruitment rates are often associated with stress on reefs (Hunte & Wittenberg 1992; Ward & Harrison 1997; Dai et al. 1998; Adjeroud et al. 2007). For example, a recruitment rate ranging between 0-133 recruit per m² per year has been reported on Taiwan reefs which are affected by eutrophication and high sediment load (Dai et al. 1998; Soong et al. 2003). In addition, reduced spat density on tiles was observed in disturbed area of the Caribbean (Tomascik 1991), and the West Indies (Hunte & Wittenberg 1992). Reef degradation in Reunion has been reported since the ‘80s and is mainly due to water enrichment from urban and agricultural pollution and coastal development (Guillaume et al. 1983; Cuet et al. 1988;

Montaggioni et al. 1989; Naim 2006; Tourrand et al. 2013). These anthropogenic factors may reduce the fitness of the adult coral colonies and lead to decrease fecundity and larval production (Ward & Harrison 2000; Loya et al. 2004). Lower fecundity was indeed observed in two coral species in Reunion compared to their South African counterparts (Chapter 1). In addition, nutrient enrichment causes the development of fleshy and turf algae that compete for space and light with corals (Cuet et al. 1988; Littler et al. 2006; Naim 2006) and may strongly limit coral settlement (Tomascik 1991; McCook 2001; Birrell et al. 2005). The study sites for the recruitment survey were situated on the reef slope of Reunion, which is less affected by water pollution and other anthropogenic pressures (Cuet et al. 2006; Cuet et al. 2011). Nevertheless, the ground water discharges and riverine outputs may spread enriched water on the reefs (Joint, pers. com.).

2. Taxonomic composition of recruit

Pocilloporidae were the dominant spat at the two localities (62 and 73% respectively in South Africa and Reunion, Fig. 7). Acroporidae were the second most dominant in South Africa (28%), was and the Poritidae (18%) in Reunion (Fig. 7). These differences in taxonomic composition of recruits between the two regions were, however, not significant, despite being consistent between years and study sites (Table 19). Coral recruitment was highly seasonal in the two regions with the overall abundance of spat approximately four times higher in summer than in winter (**Erreur ! Source du renvoi introuvable.**). This was mainly due to the contribution of the Acroporidae and Poritidae during summer. The tile surface (grooved or flat) had little influence on the taxonomic composition or seasonal variation of coral spat. The grooved tiles collected significantly more Poritidae than the flat ceramic tiles, nevertheless this concerned a limited number of spats as poritid represented represented 3-5% of the total count in South Africa.

The taxonomic composition of the coral recruitment in South Africa was consistent with previous studies on coral recruitment made at this locality (Glassom et al. 2006; Hart 2012, Table 6). It showed however a different pattern than this reported on the high-latitude reefs of the Solitary Islands (Harriott & Banks 1995) or Lord Howe Island (Harriott 1992) that had a lower proportion of Acroporidae (~12%) and a higher proportion of Poritidae (~15%, values averaged from Harriott 1992; Harriott & Banks 1995). It was also different from the Great Barrier Reef where settlement on tiles was dominated by Acroporidae (85%) and showed low proportion of Pocilloporidae (~2%) or Poritidae (~3%, values averaged from Wallace 1985c;

Fisk & Harriott 1990). The taxonomic composition of recruit on the settlement tiles seemed to reflect the local composition of coral communities. Acroporidae (*Acropora* spp and *Montipora* spp) and Pocilloporidae are among the most abundant hard corals on the reefs while the proportion of Poritidae remained low (Celliers & Schleyer 2008; Schleyer et al. 2008b).

The taxonomic composition of spat in Reunion contrasted markedly with studies at other tropical latitudes such as the Great Barrier Reef (Wallace 1985c; Fisk & Harriott 1990) and Japan (Nakamura & Sakai 2010) where Acroporidae are by far the most abundant spat on settlement tiles. Nevertheless, it was similar to the reefs in French Polynesia (Gleason 1996; Adjeroud et al. 2007), Taiwan (Soong et al. 2003) and Kenya (Mangubhai et al. 2007) that are characterised by strong anthropogenic pressures and commensurately low rates of acroporid settlement. Since Acroporidae are sensitive to perturbation at both the juvenile (Ward & Harrison 1997; Negri et al. 2007; Nozawa & Harrison 2007) and adult stage (Ward & Harrison 2000; Loya et al. 2001; Celliers & Schleyer 2002), little settlement by this family may be an indicator of stress.

In Reunion, the eutrophication of reefs has caused a shift in coral populations previously dominated by *Acropora* spp. and now by *Porites* spp. and *Montipora* spp. (Naim 2006; Bigot 2008). Such a shift may be due to the combination of direct mortality, and insufficient recruitment (Richmond 1993; Hughes & Tanner 2000) of the sensitive *Acropora* spp. (Ward & Harrison 1997; Ward & Harrison 2000; Loya et al. 2001). In contrast, high survival and successful recruitment of *Porites* sp and *Montipora* sp may be indicative of greater resilience in these corals (Banner 1974; Tomascik 1991). This shift in coral population was reflected on the settlement tiles in Reunion as Poritidae were the second most abundant spats (18%) and the recruitment of Acroporidae (4 %) was low. Nevertheless, *Montipora* sp also belong to the family Acroporidae, but this genus may not recruit on settlement tiles. The identification of recruit is limited to the family level and does not allow differing between the spats of *Acropora* or *Montipora* sp.

3. Settlement position

No differences were observed in the position of spat on settlement tiles in South Africa and Reunion. Most recruitment occurred on the upper surface of the grooved settlement tiles in the both regions. This result was expected at high latitude because of the reduced light intensity compared to the tropics, as well as intense competition with temperate biota

(Harriott & Banks 1995; Fairfull & Harriott 1999; Hughes et al. 2002; Glassom et al. 2006). It is, however, in marked contrast with the observations made in tropical areas where most recruitment occurred on the edge or on the underside of settlement tiles (ref). On tropical reefs, this pattern was suggested to develop as a result of a combination of intense grazing, competition with turf algae and sediment deposition (Birkeland et al. 1981; Penin et al. 2010; Penin et al. 2011).

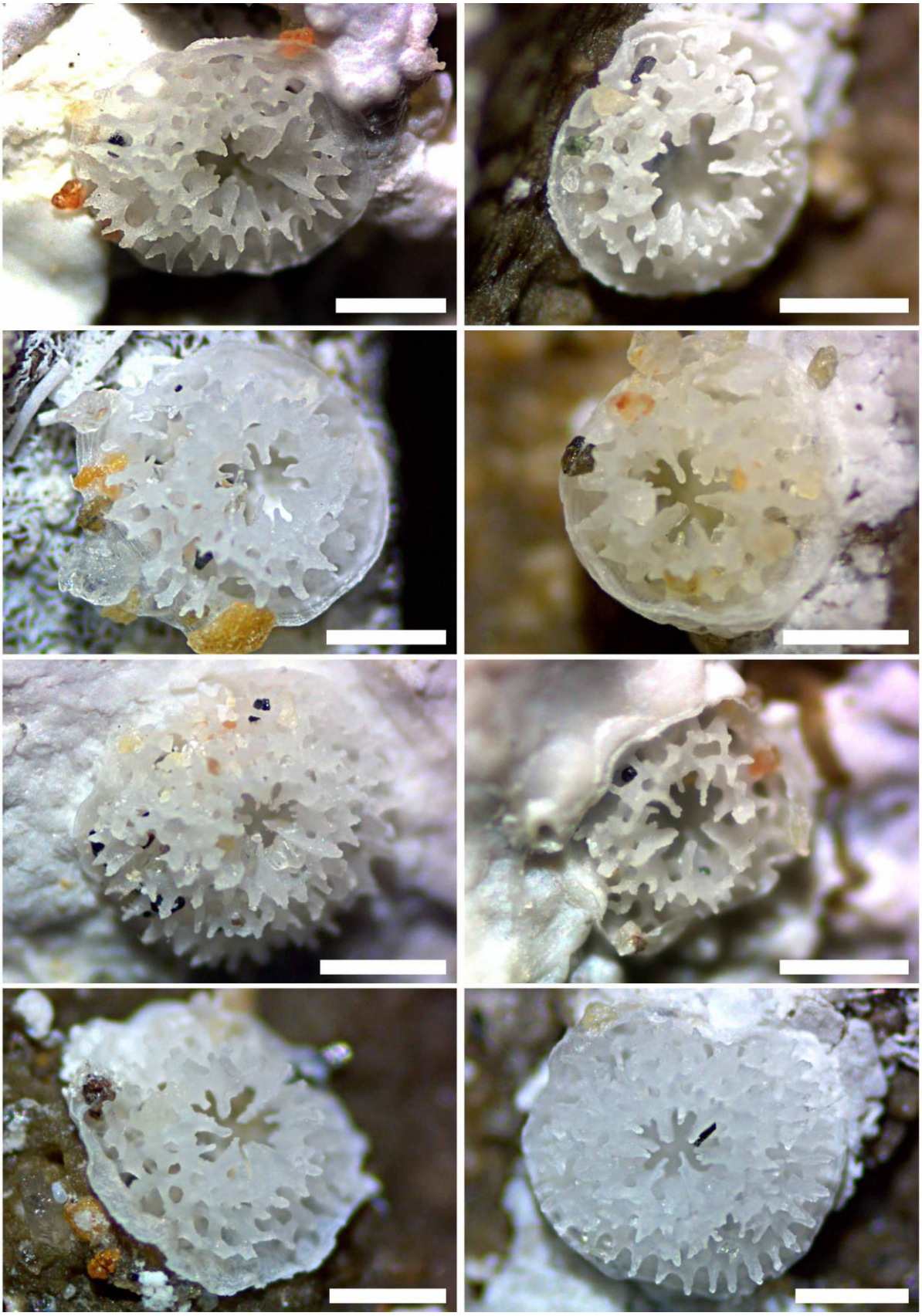
In this study, the tile design had a strong influence on the settlement position of coral spat. Significantly more spat settled on the upper surface of the grooved tiles and on the edge of the flat tiles. The tile design may therefore have more effect on the settlement position of spat than the latitudinal factor. On the grooved settlement tiles, most spat (95%) settled on the vertical edges of the grooves while only 4% settled on the horizontal upper surface between the ridges (the remaining 1% settled at the bottom of the grooves). The low number of spat on the top of the grooves suggests that grazing and competition with other biota on an exposed surface is not limited to tropical reefs.

Table 22: Recruitment rate and family composition of recruits on settlement tiles on South African and Reunion coral reefs.

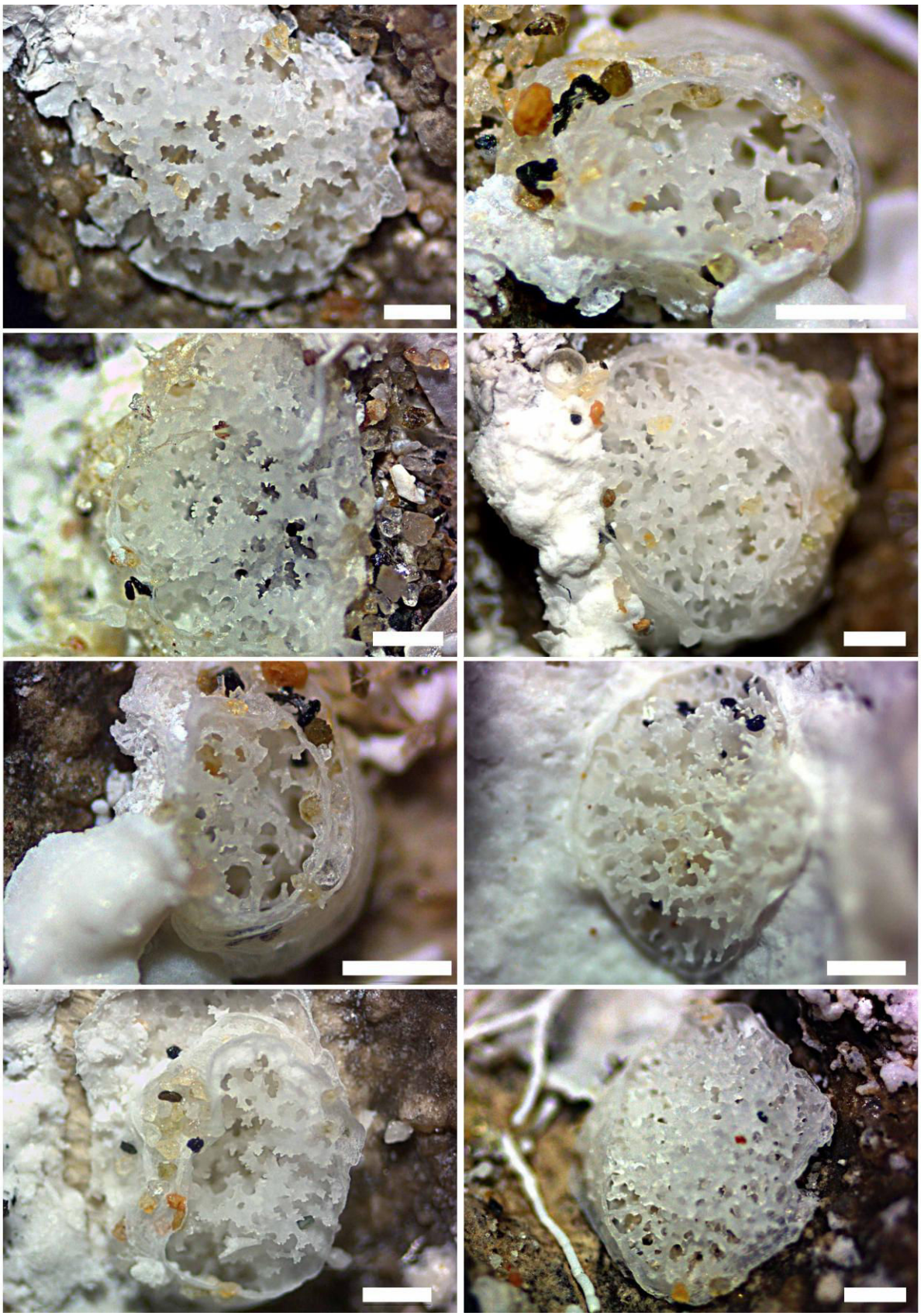
Location	Latitude	Immersion time (month)	Recruitment rate (nb m^{-2} $year^{-1}$)	Family				Source
				Acroporidae (%)	Pocilloporidae (%)	Poritidae (%)	Other (%)	
Japan, Amakusa	32°N	3	2	50	0	0	50	Nozawa *et al*. (2006)
Tanzania	5°S	2	190-374	n/a	n/a	n/a	n/a	Nzali *et al*. (1998)
	6°S	2	131	44	53	3	n/a	Franklin et al. (1998)
Kenya	3-4°S	3	101-908	0	94	3	3	Mangubhai *et al*. (2007)
Western Australia, Great Barrier Reef	15-16°S	4.5	2044	80	2	4	14	Fisk and Harriott (1990)
	18°S	4	n/a	89	2	2	7	Wallace (1985a)
French Polynesia, Moorea	17°S	4	131	5	52	38	5	Gleason (1996)
		3	40.77	12	60	18	10	Adjeroud *et al*. (2007)
Reunion	21°S	**6**	**305**	**4**	**73**	**18**	**5**	**Present study**
Japan, Iriomote Island.	23°S	5	930	70	13	12	5	Nakamura and Sakai (2010)
Taiwan	24°S	1-2.5	0-133	5	95	0	0	Soong *et al*. (2003)
South Africa	27°S	**6**	**548**	**28**	**62**	**3**	**8**	**Present study**
		variable	278-916	35	57	1	7	Glassom *et al.* (2006)
		6	653	10	88	1	1	Hart (2012)
Western Australia, Houtman Albrohos Island	29°S	3	17	83	15	0	2	Harriott and Simpson (1997)
Eastern Australia, Solitary island	30°S	3-5	132	11	62	14	15	Harriott and Banks (1995)
Western Australia, Lord Howe Island	31°S	2-4	1077	13*	64	16	7	Harriott (1992)

Appendix

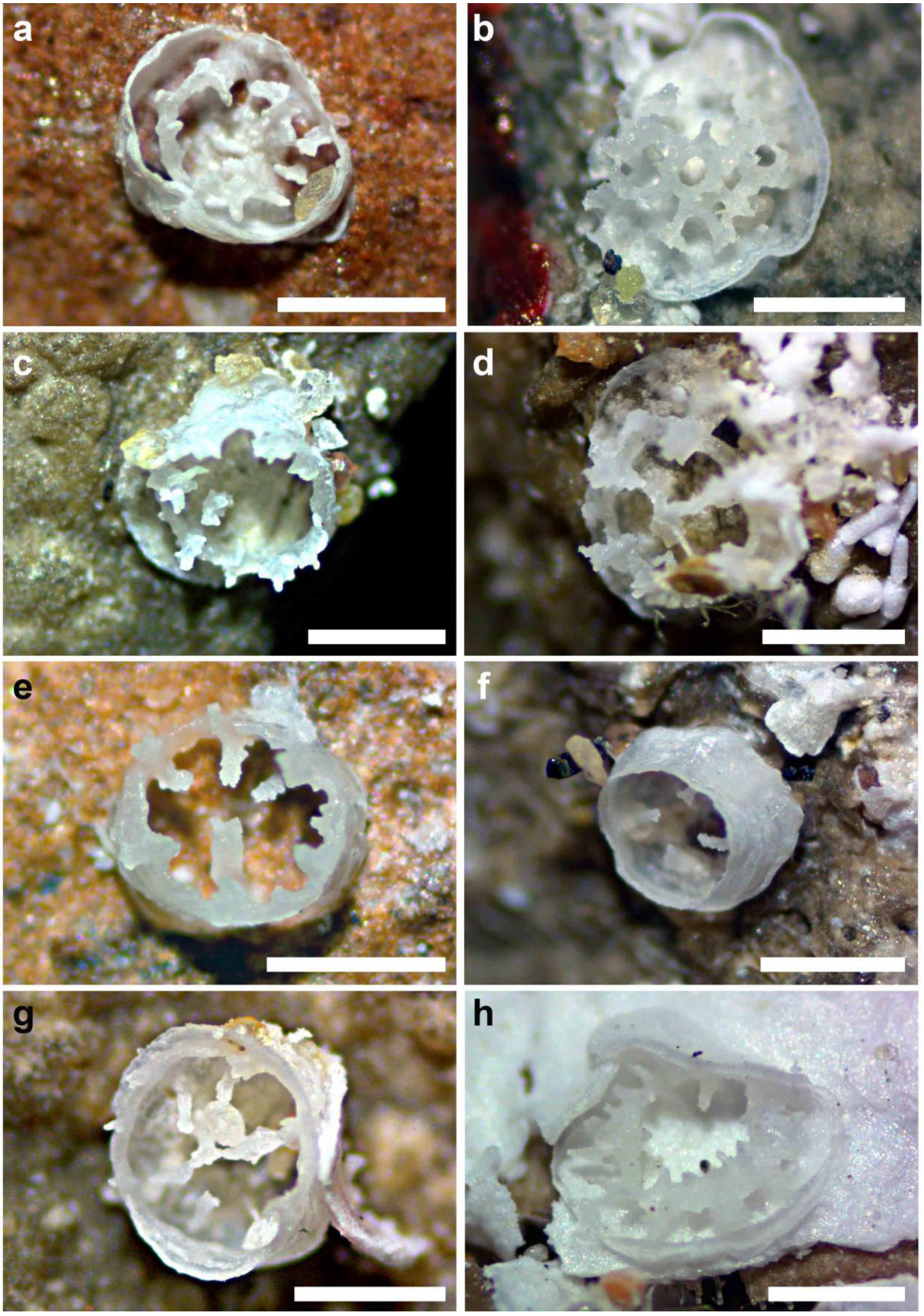
Appendix 5: Skeletal features of coral spats from the family Acroporidae. Scale bars are 500µm.



Appendix 6: Skeletal features of coral spats from the family Poritidae. Scale bars are 500µm.



Appendix 7: Skeletal features of unindentified coral spats (catgory: Other). a: early skeleton print of Acroporidae? b: unknown morphology; c:early print lacking skeletal feature juveniScale bars are 500µm.



Chapter 3:

Influence of temperature on the early-life of two subtropical corals

Introduction

Reef-building corals are limited to a narrow range of environmental variables that make them particularly sensitive to change in the environment. Optimum temperature for coral appears to be in the high twenties (25-29°C), a few degrees below the summer maxima on most tropical reefs. The increase in seawater temperature predicted in the context of global warming is therefore a major threat to coral reef (Hoegh-Guldberg 1999; Hughes et al. 2003). The recent estimated for temperature increase by 2100 (1.8-4.0°C, IPCC 2007) strongly suggests that the temperature regime on the reef will exceed the tolerances of corals (Hoegh-Guldberg 1999). This may lead to the loss of live corals unless they adapt (Day et al. 2008), acclimatise(Gates & Edmunds 1999; Coles & Brown 2003), or shift their geographical range (Greenstein 2008).

Increase in seawater temperature above the coral upper thermal limit has shown to have severe impacts on adult colonies. It may caused bleaching, reduced growth and diminished survival (see for example Brown 1997; Hoegh-Guldberg 1999; Loya et al. 2001; Baird & Marshall 2002; Mendes & Woodley 2002a). In addition, the coral early-life stages are also sensitive to high temperature that may impair embryogenesis (Bassim et al. 2002; Krupp et al. 2006; Negri et al. 2007; Randall & Szmant 2009b), larval development (Wilson & Harrison 1997; Edmunds et al. 2001; Bassim & Sammarco 2003; Baird et al. 2006; Negri et al. 2007; Randall & Szmant 2009a) and settlement (Nozawa & Harrison 2000; Nozawa & Harrison 2007). The coral early-life stages are of critical importance to ensure the replenishment and recovery of coral population. It is therefore important to understand the physiological response of corals to temperature, in particular during the early-life stages, to assist with conservation efforts.

Most studies investigating the effects of elevated temperature on the early-life of corals have focused on embryogenesis and larval development but have stopped short after settlement (see for example Nozawa & Harrison 2000; Edmunds et al. 2001; Bassim & Sammarco 2003, reviewed in Table 1, Appendix A). There is, however, a growing need to investigate coral development and survival during the first year of life to elucidate coral population dynamics and assist with reef rehabilitation (Harrison 2011; Guest et al. 2013). Working on the planktonic larvae and indiscernible primary polyps is nevertheless difficult. The recent progress in coral husbandry techniques has allowed the maintenance of corals in the laboratory from the egg to the primary polyp (reviewed in Guest et al. 2013). This provides

the opportunity to obtain a comprehensive picture of the morphological changes that occur and survival from spawning to juvenile polyps.

Heyward and Negri (2010) noted that the negative effects of increasing seawater temperature on the coral early-life occurred at similar temperature to the bleaching of adult corals. On the subtropical reefs of South Africa, corals are exposed to lower regimes of temperature than in the tropics (Schleyer & Celliers 2003) and showed a lower bleaching threshold than their tropical counterparts (c.a. 28°C, Celliers & Schleyer 2002). They may therefore respond differently to change in seawater temperature than their tropical counterparts. Studies on the effect of temperature on subtropical corals have shown negative impact on coral development from 28°C (e.g. Goniastrea australensis and Acanthastrea lordhowensis, Wilson & Harrison 1997) while some tropical coral larvae may develop up in waters heated to 34°C (Nozawa & Harrison 2007). This suggests that the subtropical corals may show some degree of acclimatisation to the local temperature regime and may be already close to their upper thermal limit (Coles et al. 1976; Wilson & Harrison 1997; Fitt et al. 2001; Celliers & Schleyer 2002).

In this study we examined the effect of temperature on the embryogenesis, larval development, settlement and juvenile phase of two coral species *Acropora austera* and *Platygyra daedalea* occurring on the subtropical reefs of South Africa. In particular, the aims were to (1) document the early life development in the two subtropical corals, and (2) assess the effect of temperature on development rate and survival of the corals from the fertilised egg to a five-month old juvenile polyp.

Materials and method

1. Temperature treatments

Two temperature gradients were applied to *A. austera* and *P. daedalea* based on their sensibility to heat and bleaching threshold. Ambient temperature on the South African reef during summer averaged 26°C; this temperature was therefore chosen as the control treatment. Bleaching in acroporid is reported from 28°C on the South African reef (Celliers & Schleyer 2002). This upper limit of thermal tolerance was used as the warmest treatment for *A. austera* and the colonies were exposed to a gradient temperature of 24, 26 and 28°C. Favid corals have not been observed bleaching on the South African Reef, therefore the effect of a warmer temperature treatment (30°C) was tested on *P. daedalea* and the colonies were exposed to 26, 28 and 30°C.

2. Collection of eggs

Ten colonies of *A. austera* and *P. daedalea* were collected in February 2011 and 2012 from Two-mile Reef (TMR, 27°31'22.56"S, 32°41'10.86"E) few days before the predicted date of spawning, as described in the materials and method of Chapter 1. They were placed in aquarium and monitored for spawning from dawn each night over 10 days. Spawning occurred over several nights in the two species but only gametes collected on the 24th February 2011 in *A. austera* and the 10th February 2012 in *P. daedalea* were used in this experiment to ensure consistency in gamete development. During spawning, the water circulation in the aquaria was turned off and the floating gametes were skimmed off the water surface using a beaker and a 4 ml pipette. They were placed in 5 l containers and gently stirred for 2 h to maximise fertilisation.

3. Larval development

The experimental design for larval breeding under each temperature treatment consisted of three replicate kreisels (2.5 L), which were floated in one 500 l water bath (Fig. 1 A and B). The water bath was maintained at the required temperature using electronic heaters or a cooler unit (accuracy ± 0.1°C) under a natural light with water recirculation (5% water replacement per hour). The kreisels were improved from the design developed by the SECORE project (SExual Coral REproduction, http://www.secore.org) and the Planugwa workshop, Guadeloupe (Oceanopolis, Aquarium of Guadeloupe, August 2010) which was based upon the plankton kreisel first developed by Hamner (1990). They consisted of plastic bowls with

holes covered by nylon mesh (150-200 µm) for water circulation (Fig. 1 C). Five water inputs (four at the top and one at the bottom) were provided to generate water motion in the kreisel. The water inputs created a circular current that aimed to limit the adhesion of coral embryos to the edge of the kreisel.

A total of 625 and 2000 eggs were counted from *A. austera* and *P. daedalea* and placed in each replicate kreisels. This corresponded to an initial concentration of 25 and 80 embryos per 100 ml in *A. austera* and *P. daedalea* respectively in each kreisel. The remaining eggs were held in additional kreisels for use in the settlement experiment. The development and survival of coral embryos was ascertained by the regular removal of water samples from the three experimental kreisels for observation under a stereomicroscope, until their development into a competent planula. Three repetitive samples of 30 ml were collected at each time in the replicate kreisels. Survival was calculated in terms of the number of live embryos per 100 ml; dead or deformed (showing developmental aberration) embryos and planulae were counted separately. The size of each e

mbryonic stage was determined by photographing 7-20 individuals with a Zeiss AxioCamERc5s camera with AxioVision 4.8 software and measured the longest diameter to the nearest 0.01 mm.

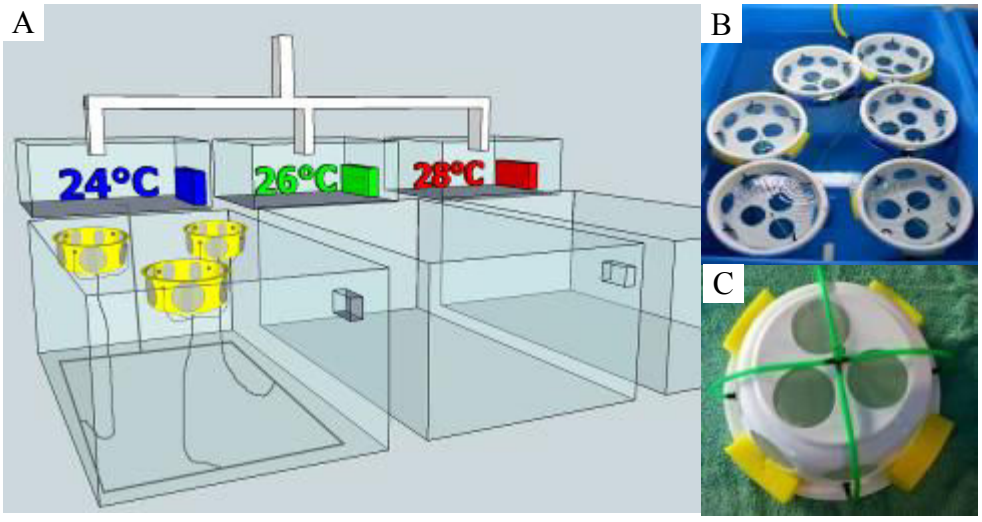


Figure 31: Experimental design for the brooding of coral larvae under different temperature regimes. A: System design; B: view of replicate kreisels in a tank; C: underneath view of a kreisel showing the 5-water inputs.

4. Settlement and juvenile development

Three days after spawning (DAS), planula larvae were harvested from the kreisels and spread equally in three 100 l aquaria containing five horizontal ceramic tiles (10 x 10 x 1 cm) for settlement (Figs. 2 and 3). Meanwhile, approximately 30 larvae were isolated in a one-litre beaker with a settlement tile and observed under a stereomicroscope to document settlement behaviour and metamorphosis. The settlement tiles were conditioned in an open-water aquarium for at least three months prior to their use to provide as natural a settlement substratum as possible. UV-sterilised seawater was pumped (5% replacement per hour) to the aquaria and maintained at 26°C with a heater. Water parameters (salinity, pH, nitrate/nitrite) were checked on a regular basis and maintained at the optimal levels indicated by Petersen *et al*. (2008). The planulae were prevented from entering the pump and water outlet by a mesh screen (250 µm) to prevent their loss. The aquaria were exposed to muted daylight (light intensity: 40 µmol m² s^{-1}) until the acquisition of zooxanthellae by juvenile polyp. Artificial light was initially excluded to limit the growth of algae, which otherwise rapidly overgrows coral juveniles (Petersen et al. 2008). Artificial light, comprising two white and two blue bulbs (HAILEA®LFHO LAMP, 4x 54 W) above the aquaria, was introduced one month after spawning (8 h light per day at 140 µmol m² s^{-1}). The tiles were regularly examined for coral settlement under the microscope. Each new coral was mapped and numbered (Fig. 3) to document its survival and development over time. Tiles on which corals had developed and subsequently died were collected and cleaned with household bleach for examination of the skeleton print.

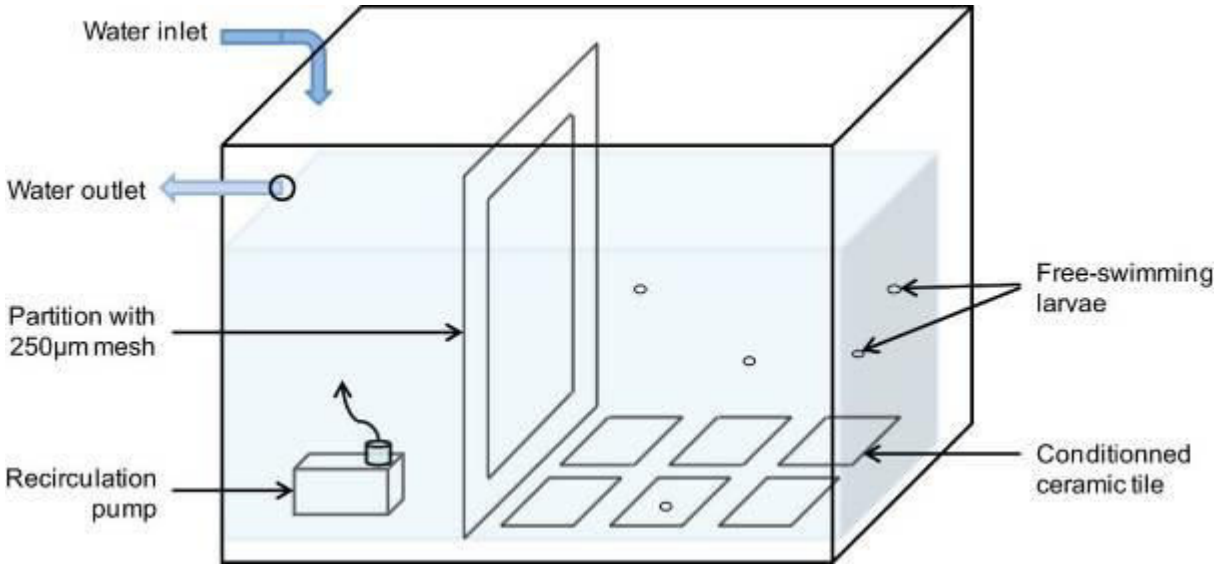


Figure 32: Experimental design for the settlement of coral under three regimes of temperature.

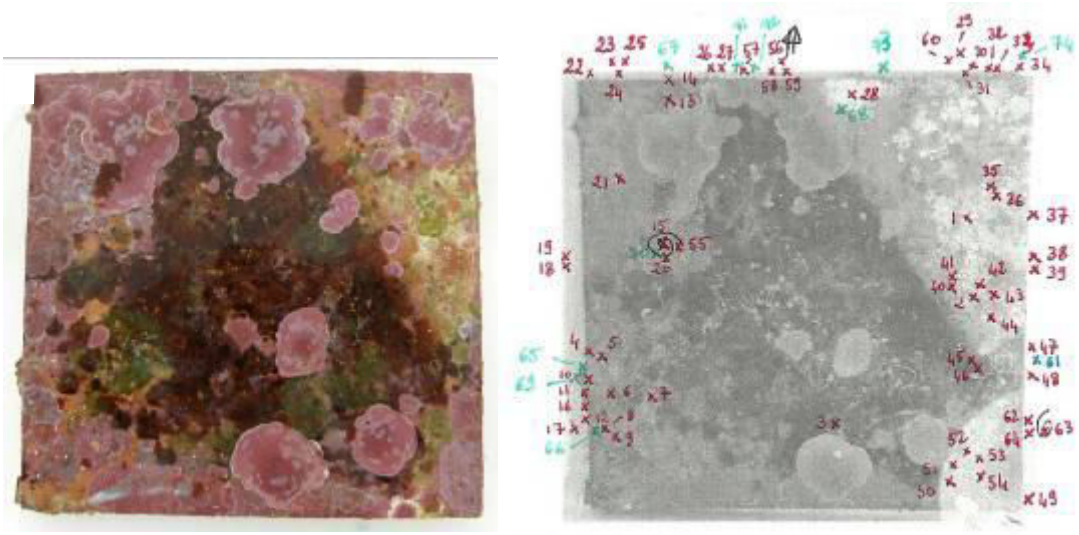


Figure 33: Coralline algae-conditioned ceramic tile used for settlement surveys in aquaria. A: Overall view of the tile (10x10 cm); B: map of the settled corals on the tile, 15 days after the beginning of the experiment. The red numbers indicate corals settled during the previous count and green numbers show newly-settled corals.

5. Development and survival rates

5.1. Rate of development

To ascertain the rate of development in the two studied species, several stages of larval and juvenile development were defined based on the observations made in this study and the descriptions from the literature (Shlesinger & Loya 1991; Hayashibara et al. 1997; Ball et al. 2002). A code number was attributed to each development stage. The development index (DI) was then calculated as the proportion of coral that had reached a given developmental stage using the following calculus:

$$\mathrm{DI} = \frac{\sum_{i=1}^{n} w_i x_i}{\sum_{i=1}^{n} w_i}$$

where w_i is the code number (1, 2, 3...) attributed to the development stage x_i.

Significant difference in the development rate of embryos, larvae and juveniles corals between treatments, date of sampling, and replicates were ascertained using main-effect ANOVAs.

5.2. Survival

The survival rate during the larval phase, was estimated by the Kaplan-Meier curves plotted using Prism (5.03). Significant differences in survival rate between treatments were ascertained with a Logrank Mantel-Cox test (LMC test). It was not possible to apply the Kaplan-Meier method to assess survival at the juvenile phase as the initial number of individuals varied over time with the settlement of new corals. Survival during this phase of development was therefore compared using a main-effect ANOVA. All ANOVAs were performed on Statistica 10.0 (Statsoft, 2011).

6. Settlement preference

A variety of settlement surfaces was available on the preconditioned tiles. They were classified into seven easily recognisable categories and the type of substratum on which the coral had settled was noted. In addition, its position on the tile (upper surface of edges) was recorded. The influence of temperature on the settlement preference (type of substratum and position) of coral spat was tested using Main-effect ANVOAs.

Results part 1:

Description of the early-life development in

Acropora austera and Platygyra daedalea

Acropora austera

1. Embryogenesis

The timing and development of embryos and larvae in *A. austera* are summarised in **Table 23**. The fertilised eggs in *A. austera* were pink and measured approximately 500-700 µm. The first cleavage started rapidly i.e. 2h after fertilisation and the morula was observed from 6 h after spawning. It was a pink pile of big rounded cells. The blastula stages occurred between 10-15h. During this phase, the embryos developed from a prawn-chip and to a donut shape as described in the development of other Acropora (Hayashibara et al. 1997). A round gastrula was observed 24 h after spawning. Unfertilised oocytes were observed for the last time 10 h after fertilisation. They were degraded over time and released their lipid droplets that accumulated at the water surface in a greasy layer.

Table 23: Timing of larval development in *A. austera* in South Africa

	Time after spawning	Stage of development	Maximum length (µm)
Embryogenesis	2 h	First cleavage	700
	6 h	Morula	690
	10 h	Early blastula (prawn-chip shape)	780
	15 h	Late blastula (donut shape)	590
	24 h	Gastrula	590
Larval development	1 d	Mobile planula (pear shape)	690
	2 d	Elongate planula sinking	840
	4 d	Elongate planula with visible oral pore	
	21 d	Last observation of live planula in the kreisels	

2. Larval development

A round shape motile planula was first observed at 1.5 days after spawning (Fig. 4 a). It swam rapidly in all directions and remained mostly buoyant in the kreisels. The planula became then elongate in the oral-aboral axis at two days after spawning (Fig. 4 b). Its shape was various as it could contract and change its morphology while swimming. In the late planula, the pharynx has formed, leading inward from the oral pore (Figs 4 c and d). The pore eventually becomes the mouth of the planula larva.

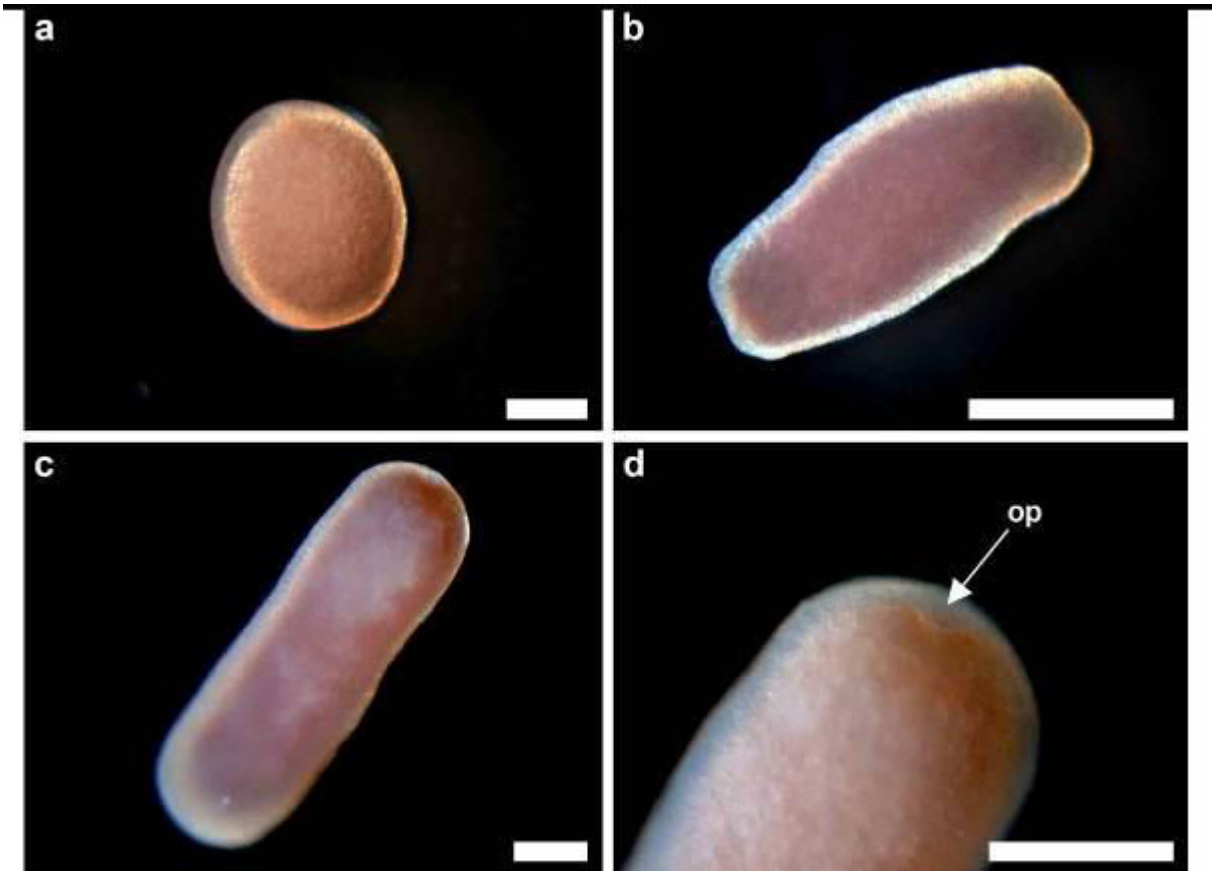


Figure 34: Larval development in *A. austera* of South Africa. a: early planula at 1.5 days after spawning; b, elongate planula at 2.5 days after spawning, c and d, elongate planula with a blastopore at 4 days after spawning. Scale bar is 200µm in a, c, d and 500µm in b. op, oral pore

Platygyra daedalea

1. Embryogenesis

P. daedalea released bundles of oocytes and sperm when spawning (Fig. 5 a), which broke up at the water surface to release the gametes. The bundles sometimes remained intact at the water surface for 1-2 h in the absence of water movement (Fig. 5 b). The oocytes were spherical (490 μm, Table 24) and azooxanthellate. Egg development started 30 min to 1 h after fertilisation with the first cleavage of the oocytes. Four to six hours after spawning, the embryos had flattened and developed into morulae (520 μm) slightly bigger than the oocytes. Each morula was composed of an irregular arrangement of blastocoels which broke apart fairly easily and the loss of cells led to the death of the embryo. Two to four hours later, the embryos were at the early blastula stage composed of two cell layers (Fig. 5 d). These were "prawn-chip" in appearance with a clear centre. The early blastula then thickened and formed a depression on one side where the edges folded upward (Fig. 5 d). This gave the late-blastula stage a donut shape (Hayashibara et al. 1997; Ball et al. 2002). Invagination of the endoderm continued and ended with the development of a gut cavity closed by a pore at the gastrula stage, 16-18 h after fertilisation (Fig. 5 e). The gastrula (460 μm) was slightly smaller than an oocyte and was round in shape. Some had cilia 19 h after spawning and had become motile.

Figure 35: Spawning, embryogenesis and larval development in Platygyra daedalea. a: Polyps releasing egg-sperm bundles; b: egg-sperm bundles showing compacted oocytes (pink) and sperm (white); c: embryos at the blastula stage 8 h after spawning; d: embryos at different development stages 12 h after spawning; e: gastrula 18 h after spawning; f: spindle-shaped planula 5 d after spawning; g and h: 12 d old planula showing initiation of metamorphosis; i: attachment and initiation of metamorphosis in 12 d old planulae. Bl: blastula; do: donut-shaped blastula; mo: morula; mu: mucus. Scale bars, a: 1 mm, b-i: 200 µm.

Table 24: Development of *Platygyra daedaea* from embryogenesis to juvenile polyp in South Africa.

	Time after spawning	Stage of development	Maximum length (μm)
Larval development	2 h	First cleavage	490
	4-6 h	Morula	600
	10 h	Early blastula (prawn-chip shape)	560
	15 h	Late blastula (donut shape)	550
	18 h	Gastrula with oral pore	490
	1 d	Mobile planula (pear shape)	410
	2-5 d	Spindle-shaped planula; sinking	700
	>7-26 d	Partial metamorphosis of planula with four visible mesenteries	530
	1 mo	Last observation of live planula	n/a
Settlement and juvenile development	7-8 d	Planula attaching and flattening	630
	9 d	Planula metamorphosis, mesenterial development and tentacle buds	660
	14 d	Early primary polyp with nine tentacles, a basal ring and first zooxanthellae	830
	24 d	Fully-developed primary polyp with nine tentacles	
	1-2 mo	Corallite wall formation with initial septa	
	3 mo	Polyp composed of twelve tentacles. Full zooxanthellae complement and early pigmentation	1700
	5 mo	Polyp composed of 18 tentacles, white pigmentation around the mouth. Primary and secondary septa clearly visible in the corallite	2030
	7 mo	Polyp composed of 24 tentacles, first budding, white and green pigmentation around the mouth, complete skeleton print	3760

2. Larval development

Pear-shaped, mobile planulae were observed 24-28 h after spawning (Table 24). On average, they were similar in size to the gastrula (460 μm). These remained in the water column where they swam rapidly in all directions. They sometimes stopped for several minutes before swimming off again. Two to 5 DAS, the planulae became elongated (Fig. 5 f), i.e. spindle-shaped (Ball et al. 2002), although they could contract and change shape while swimming. The oral pore (or blastopore) was clearly visible and faced forward during swimming. These spindle-shaped planulae were slower-moving and tended to sink. They actively searched the substratum with the oral pore end, spinning on the longitudinal axis. Some planulae started attaching to the kreisel screens from 4 DAS; however, it was still easy to remove them by gently flushing them off the netting with a pipette. Seven DAS, four primary mesenteries (edwarsia stage) were visible in the body cavity of some planulae, suggesting that metamorphosis had commenced (Figs. 5 g and h). In the meantime, two to three protrusions developed at the aboral end of the planulae but they remained highly mobile. In rare cases, the

metamorphosing planulae were observed attaching to each other with mucus in the water column (Fig. 5 i). Most of the planulae remained alive with no sign of metamorphosis until final transfer to the settlement tanks 12 DAS.

3. Settlement and metamorphosis

Early planulae were first transferred into the settlement tanks 3 DAS (13/02/12) but no settlement was observed for the ensuing three days. A further 600 planuale were then added 6 DAS and settlement was observed at 8 DAS. From 6-8 DAS, the coral larvae slowly searched the substratum (Fig. 6 a). They sometimes remained spinning in the same position for a few hours before moving on to a new location. The planulae moved freely along the top and the edges of the tiles and did not avoid the ambient or microscope light. They were observed spinning above various types of substratum e.g. bare substratum, living or dead coralline algae, or sediment. Newly-attached planulae were pink and ball-shaped. Four to six hours after attachment, they flattened, started subdividing radially along the mesenteries (Fig. 6 B) and measured 630µm (Table 24). Metamorphosis led to the formation of the polyp body, characterised by a mouth surrounded by a ring of tentacles which budded 9 DAS (Fig. 6 c). Larval settlement was patchy and up to 5 recruits per cm² were observed on some tiles; they did not seem to establish any particular distance between them. An average of 9 % of the larvae settled from 8 and 10 DAS. The peak of settlement occurred at 15 DAS when 30% of the larvae settled on the tiles. Of the 1200 larvae initially introduced to each experimental tank, an average of 643.88 ± 123.76 larvae settled per tank, which corresponds to a settlement rate of 54%. Further recruitment was observed on the tiles up to three months after spawning.

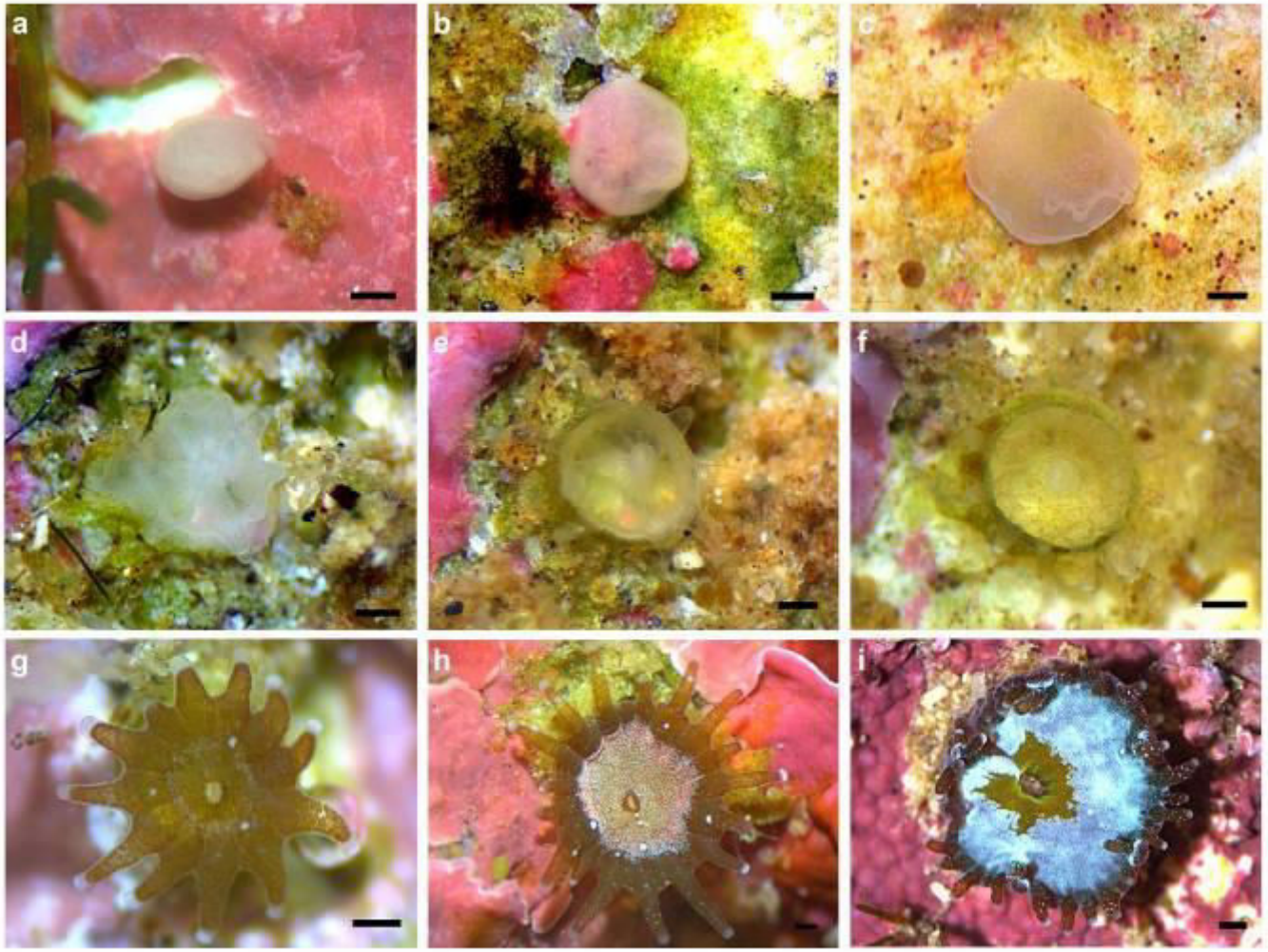


Figure 36: Metamorphosis and juvenile development in Platygyra daedalea. a: Foraging larvae searching the substratum (5 d); b: attached larvae flattening (7 d); c: metamorphosis (9 d); d: primary polyp with basal ring (14 d); e: fully-developed polyp (24 d); f: polyp with 12 tentacles (2 mo); g: complete zooxanthellae complement and early pigmentation (3 mo); h: polyp with 18 tentacles and pink pigmentation (5 mo); i: 7 mo old polyp. Ages of larvae and polyps (d: day, mo: month) are given from day of spawning. Scale bars are 200 µm.

4. Juvenile polyp development

Following metamorphosis, a whitish primary polyp appeared 12-14 DAS (Fig. 6 d). At this stage, the mouth was fully formed but the tentacles were still short. Sparse zooxanthellae were first observed from 14 DAS around the polyp mouth (Fig. 6 d). The primary polyps were fully-developed 24 DAS, 830 µm in diameter and had nine extended tentacles (Fig. 6 e). The number of tentacles increased from 9 to 12 from one to two months after spawning, and the zooxanthellae became denser in the polyp tissue (Fig. 6 f). Three months after spawning, the zooxanthellae had completely colonised the tissue of the polyps, which were 1.7 mm in diameter (Fig. 6 g). White pigmentation spots appeared at the base of the tentacles. They varied in number and showed no particular pattern. Five months after spawning, the polyps were 2 mm in diameter and had 18 short tentacles (Fig. 6 h). They had dense white pigmentation around the mouth that could be tinged with pink or green. At seven months, the polyps were 3.7 mm in diameter and their mouths were surrounded by green pigmentation (Fig. 6 i). A few polyps had started budding by dividing in two.

5. Skeletal development

Skeletal development in *P. daedalea* is illustrated in Figure 7. For the first two months of development, deposition of the calcareous skeleton was limited to a thin ring (680 µm) which was hardly visible (Fig. 7 A). As a polyp developed, its corallite wall (epitheca) grew upward to support the coral tissue. Small protrusions were first observed emerging from the basal ring of the skeletal prints two months after spawning. The early septa became evident one month later and originated from the polyp wall (Fig. 7 B). The corallum grew in size by expansion of the basal disk and septa over the substratum from four to five months after spawning (1.6 mm, Figs 7 C and D). As the septa expanded toward the centre of the corallum and the costae towards the periphery, they became ornamented with spicules (Fig 7 E). The second cycle of septa appeared at the perimeter of the basal plate, six months after spawning (Fig. 7 E) The corallites were now 2.5 mm in diameter and continued to grow to 3.1 mm seven months after spawning, at which time they had a complete and clearly recognisable skeletal print (Fig. 7 F), with six primary and secondary septo-coastae arranged radially.

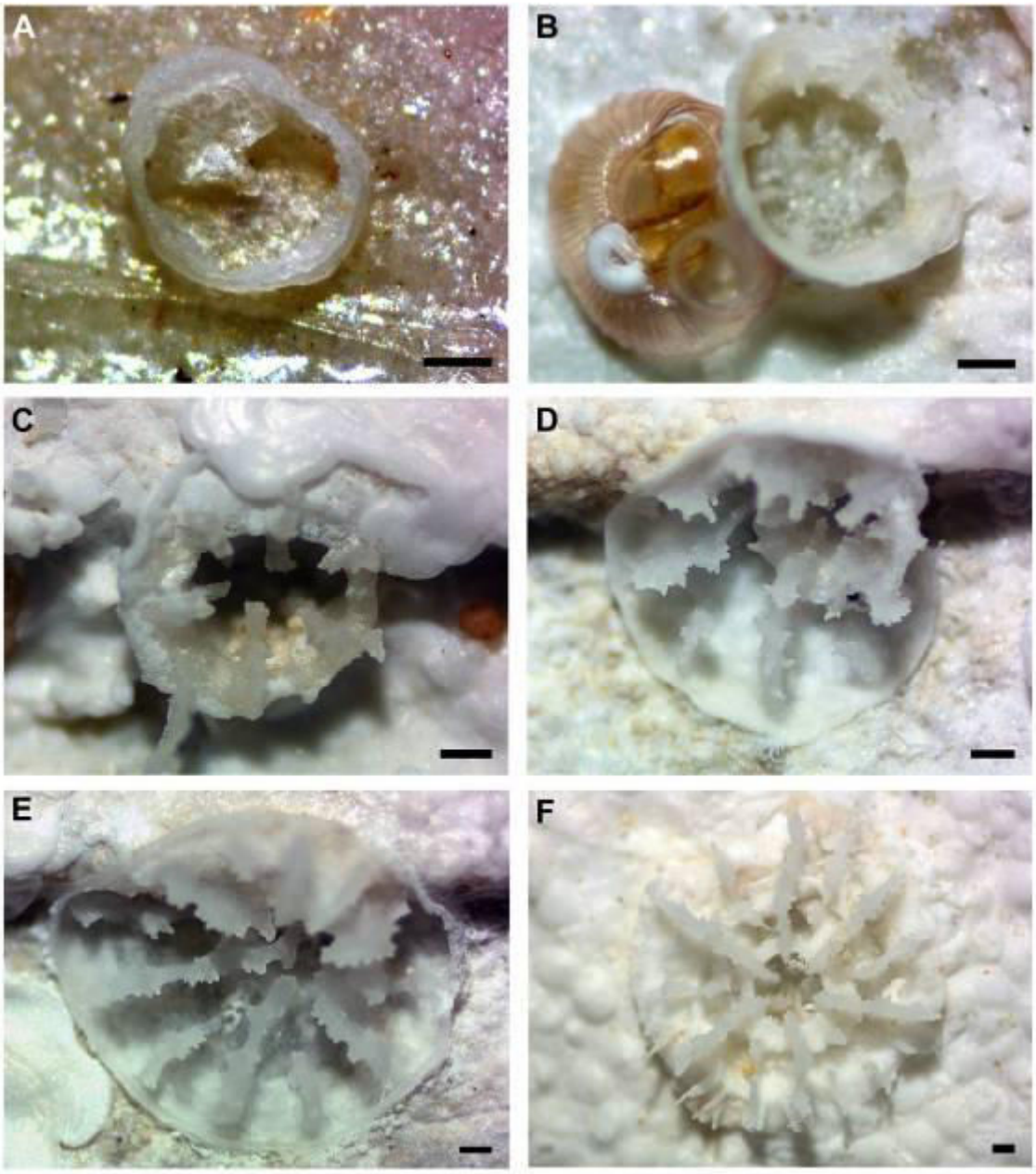


Figure 37: Skeletal development in *P. daedalea*. a: Skeleton print constituted of a single basal ring (14 days); b: basal wall and early cycle of septa (3 months); c and d: expansion of septa toward the centre and the periphery of the corallite 4 and 5 mo after spawning; e: development of the second cycle of septa at 6 mo; f: fully developed skeleton with complete cycle of septa 7 mo after spawning. Ages of the prints are given from the day of spawning. Scale bars are 200 µm**.**

6. Substratum selection

Seven types of substratum were identified on the settlement tiles and are illustrated in Figure 8. Most coral recruits (38%) settled on dead coralline algae (Fig. 8 a) covered by a thin film of filamentous green algae and microorganisms. This biofilm had also overgrown previously bare areas of the ceramic tiles (Fig. 8 b) and attracted a further 22% of the coral recruits. A high number of spat (21%) also settled on live coralline algae, a *Mesophyllum* sp. (Fig. 8 c), or in its vicinity (less than 5 mm away) but few corals settled on a mix of living and dead coralline algae (7%). Dead skeletons of tube-worms (Fig. 8 d) attracted 8% of the recruits. A brown unidentified cyanobacterium (2%, Fig. 8 e) and compacted sediment (2%, Fig. 8 f) were the least preferred surfaces for settlement. Most recruitment occurred on the upper surface of the tiles (67%) compared to the outside edges.

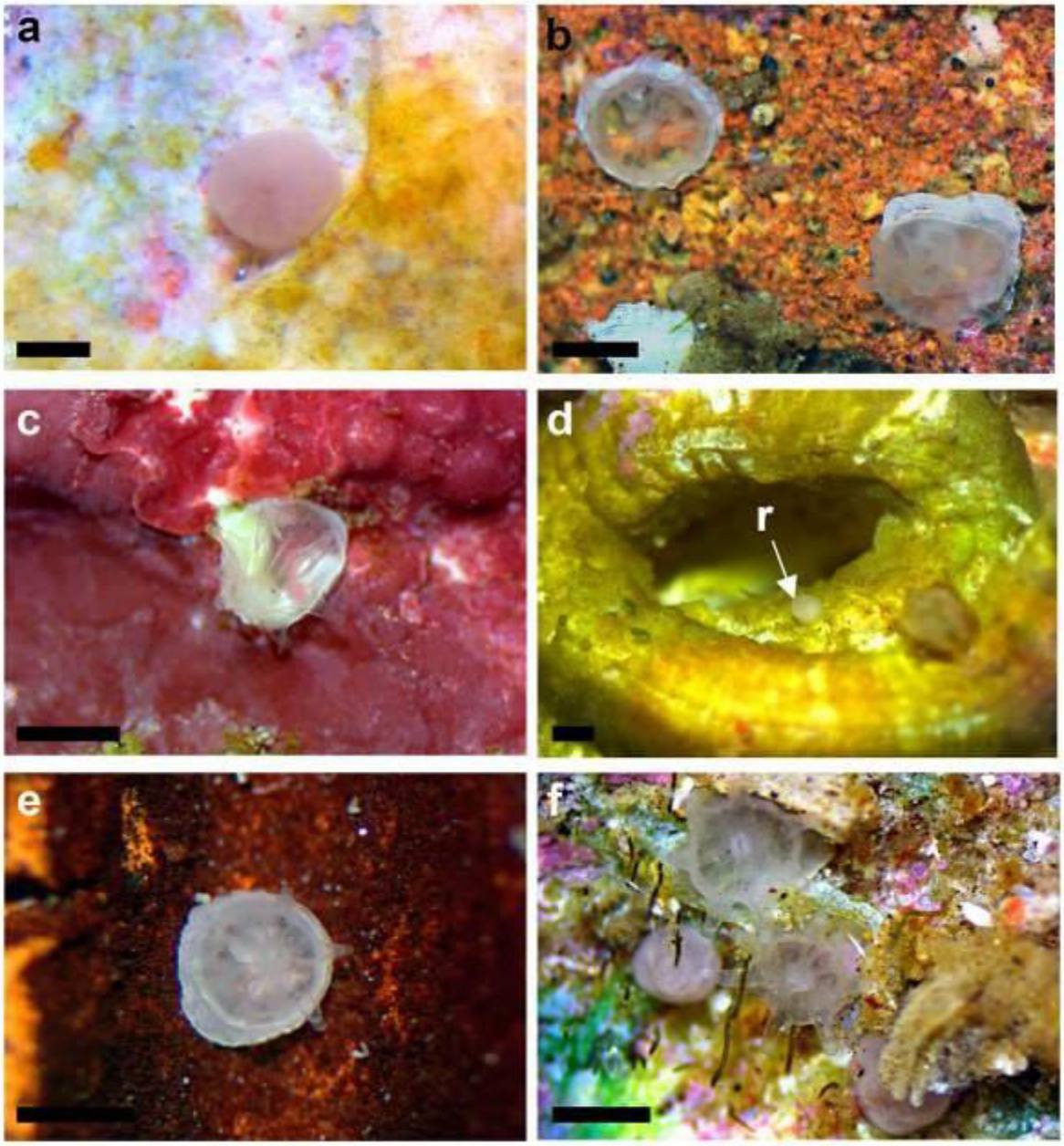


Figure 38: Main types of substrata selected by *P. daedalea* larvae for settlement. a: dead coralline algae; b: bare ceramic covered with a biofilm; c: alive coralline algae (*Mesophyllum* sp.); d: skeleton of dead animals for example tube-worm; e: unidentified cyanobacterium; f: compact sediment. r: recruit. Scale bars are 500µm.

Result Part 2:

Influence of temperature on the early-life of two subtropical corals Acropora austera and Platygyra daedalea

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Acropora austera

1. Larval development

The larval development and survival rate of embryos in *A. austera* under the three temperature treatments are shown in Figure 9 A and B respectively. The development of fertilised eggs into competent planulae was faster with increasing temperature. It took approximately three days at 28°C, four days at 26°C but required two more days to be completed at 24°C (~ 6 days in total). The rate of development was similar between the treatments for the first two days of temperature exposition *i.e* until gastrulation (Main-effect ANOVA, 0-58h, $p>0.05$). It became then significantly different between treatments (Main-effect ANOVA, $p<0.001$). The larval development was consistent within the replicates of a same treatment and no significant difference was found in this rate between the replicates (Main-effect ANOVA, $p>0.05$). Embryos with developmental aberrations were observed in low proportion (5-9% of the total number of embryos) in the three temperature treatments. No significant difference in the total number of these embryos was however observed between the temperature treatments (Main-effect ANOVA, $p>0.05$).

2. Survival

The survival rates of coral embryos in *A. austera* differed significantly between the three temperature treatments (Logrank Mantel-Cox tests or LMC, tests, 24 *vs* 26°C, 24 *vs* 28°C and 26 *vs* 28°C, $p<0.001$). On average, survival was the lowest in the warmest treatment and the highest in the coolest treatment. No significant difference in the survival rate of the embryos and larvae was found between the replicate kreisels (Main-effect ANOVA, $p>0.05$).At 28°C, the survival rate of embryos decreased rapidly from 24h after spawning. In contrast, this was more gradual in the control and coolest treatments. When the larvae became competent to settle, the survival rate at 26 and 28°C was similar c.a. 35%. In contrast, survival in the

coolest treatment averaged 7%. Twenty six days after spawning, no larvae remained in the 24 and 26°C temperature treatments while a total of 8 larvae were still swimming at 28°C. Six of these larvae had metamorphosed in the kreisels.

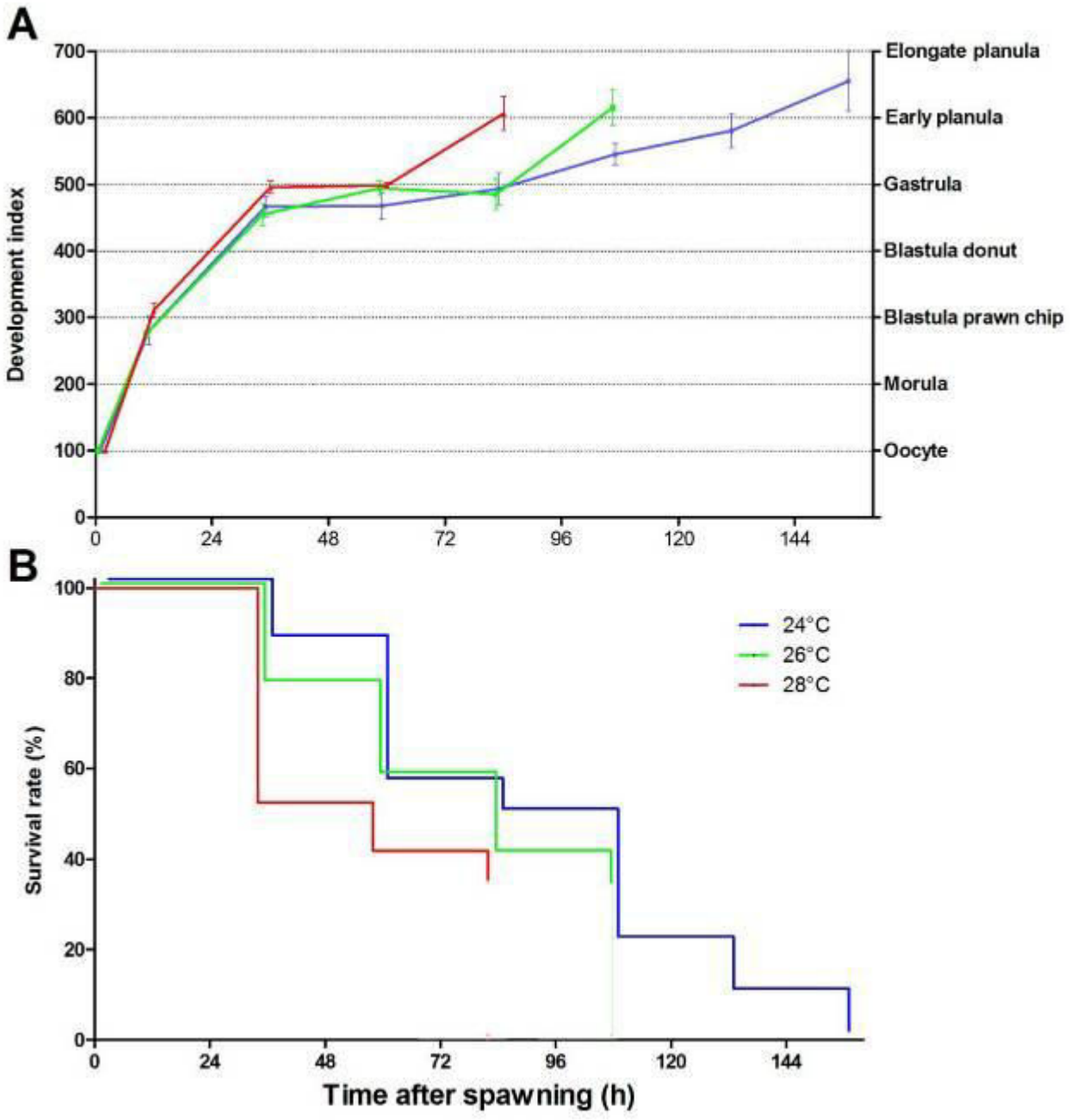


Figure 39: Larval development (A) and survival rate (B) in *A. austera* exposed to three temperature regimes. The survival curves were calculated with the Kaplan and Meier method. Graph A shows mean and standard error (se).

Platygyra daedalea

1. Larval development and survival

The rate of development and survival from embryogenesis to the larval stage are shown in Figure 10. No significant difference in the rate of larval development was observed between the three temperature treatments (Main-effect ANOVA, $p > 0.05$) but the survival rate was found to differ significantly between the treatments (Logrank Mantel-Cox, LMC test, $p<0.001$, Table 31, appendix B: 0-228 h). It was the highest at 26°C and the lowest at 30°C.

Three phases could be distinguished in the development and survival of embryos over time (Fig. 10). The embryogenesis (from fertilised egg toward a pear-shaped planula) was fast in all three treatments (~48h) and accompanied with higher rate of mortality in the warmer treatments than in the control (LMC test, 0-48 h, 26 *vs* 28°C and 26 *vs* 30°C, $p<0.001$). As the larval development slow down between 48 and 96h, a drastic drop in the survival rate was observed at 26 and 30°C; it was more gradual at 28°C. At this stage, the survival rate was still significantly higher in the control than in the two other treatments (LMC test, 48 h-96 h, 26 *vs* 28°C and 26 *vs* 30°C, $p<0.001$). From 96 h after spawning, the planula larvae became elongated and started searching the substratum by spinning on themselves. Mortality was still the highest at 30°C. It was however not significantly different between the 26 and 28°C treatments (LMC test, 96-228 h, $p>0.05$) until the end of the experiment. Ten days after spawning, early metamorphosis was observed in some larvae as shown by the presence of mesentery in their body. The total number of this larvae did not differ significantly between treatments (T-tests, $p>0.05$). A low number (1.6-1.8%) of embryos and planula larvae showing developmental aberrations were observed throughout the experiment. No significant in the abundance of these embryos was however found between the three temperature treatments (Main-effect ANOVA, $p>0.05$)

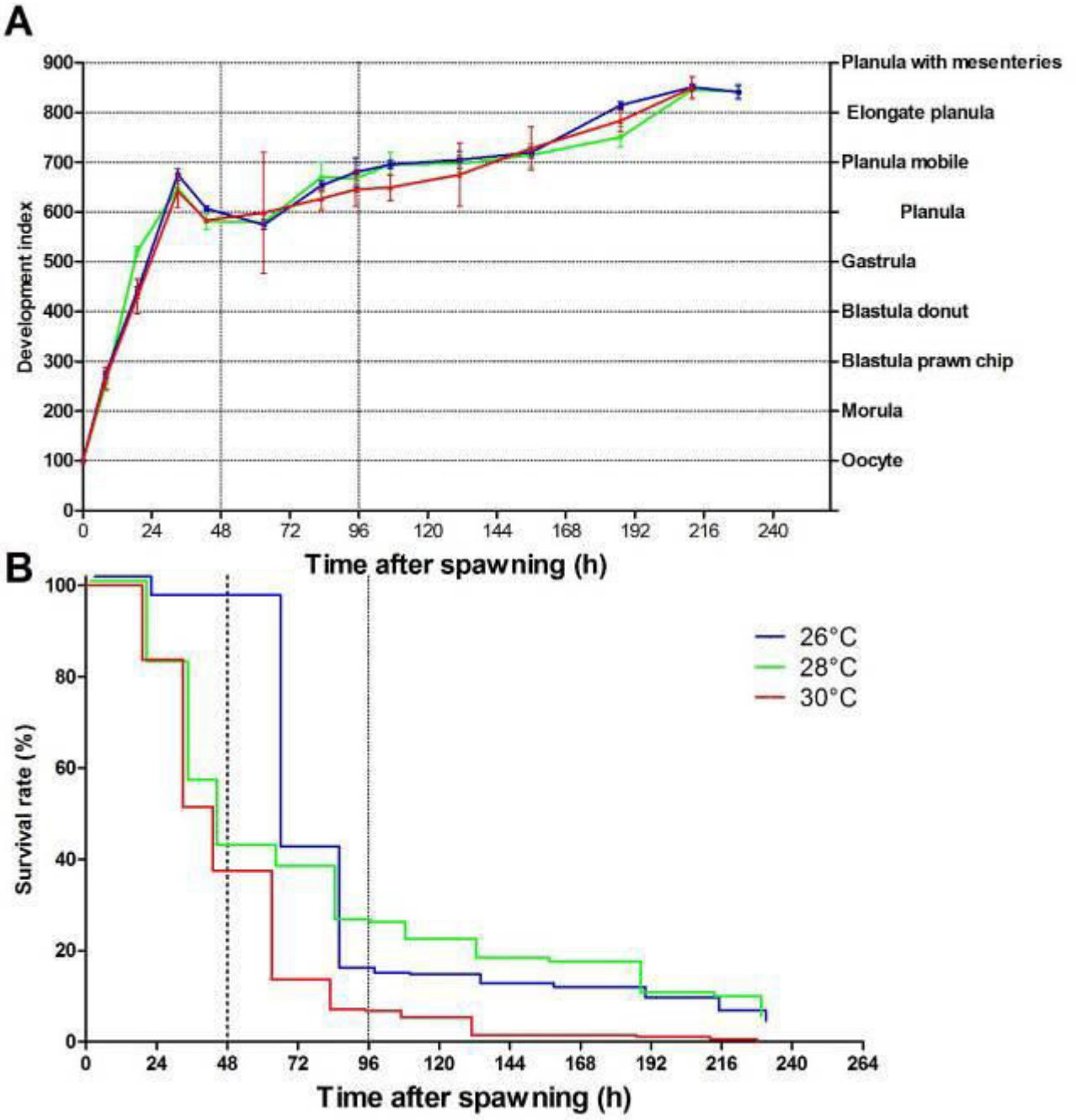


Figure 40: Larval development (A) and survival rate (B) in *P. daedalea* exposed to three temperature regimes. The survival curves were calculated with the Kaplan and Meier method. Graph A shows mean and standard error (se).

2. Settlement

2.1.Settlement rate

The settlement rate was faster at 28°C for the first days of the experiment, as indicated by the greater number of spats (50 spats) settled at this temperature compared to the 26°C (29 spats) and 30°C (21 spats) treatments (Fig. 11 A). This trend was however not maintained afterwards and the peaks of settlement were observed simultaneously in the three temperature treatments at 15 DAS. Settlement remained high at 26°C until 40 days after spawning. New settlement on tiles was observed up to 100 DAS at 26°C; but stopped earlier at 70 DAS in the heated treatments (28 and 30°C). The temperature had a significant effect on the total number of larvae settled (Main effect ANOVA, $p<0.001$, Fig. 11 B). Overall, a significantly higher number of recruit settled at 26°C (27%) compares to the 28 (16%) and 30°C (13%) treatments (Fisher LSDs, $p<0.001$). No significant difference was however found between the two warmer treatments (Fisher LSD, $p>0.05$). Settlement in *P. daedalea* was not uniform. It varied significantly between tiles and replicates (Main effect ANOVA, tiles, $p<0.001$; replicates, $p<0.05$).

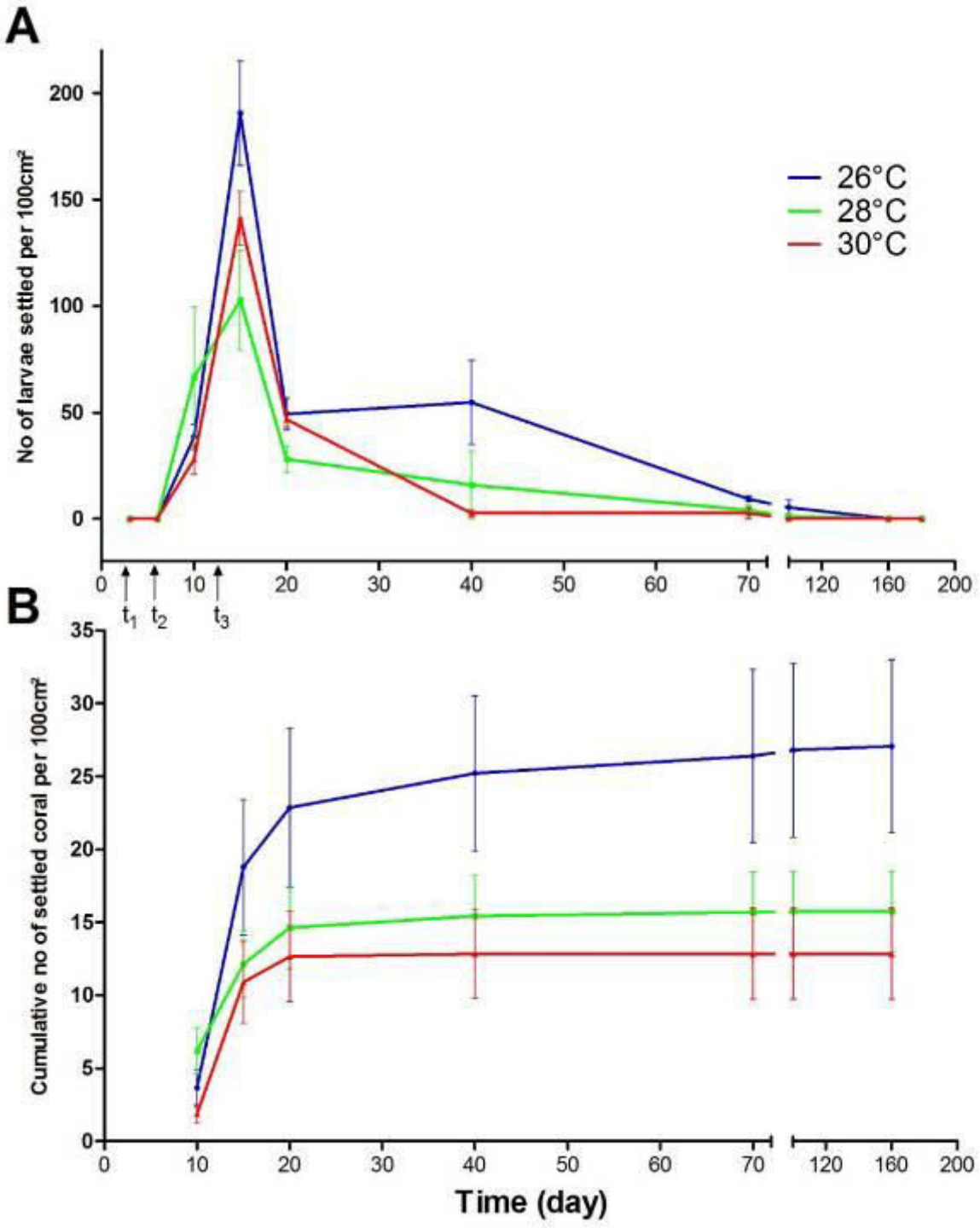


Figure 41: Settlement (A) and cumulative number of settled spats (B) in *P. daedalea* under three temperature treatments. T1, t2 and t3 correspond to the addition of larvae at 3 (N = 400), 6 (N = 600) and 12 (N = 200) days after spawning. The graphs are showing average ± SE.

2.2.Substratum selection and settlement orientation

A variety of settlement surfaces was found on the preconditioned tiles. They were grouped onto 7 categories to characterise the settlement preference of the coral larvae and are illustrated in Figure 8. In the three temperature treatments, most settlement occurred on the skeleton of dead CCA (28-33%), followed by the biofilm that had grown on the bare ceramic (18-24%), and on the CCA *Mesophyllum* sp.or in its vicinity (less than 5 mm away 10-17%, Fig.12). No significant difference in the number of larvae settled on these three substrata was found between the temperature treatments (Main-effect ANOVA, $p>0.05$). In contrast, significant difference in the settlement preference of larvae between treatments were observed for the last four categories of substratum (Fisher LSDs, $p<0.05$). A total of 11% of larvae settled on a brown cyanobacterium at 30°C but this trend was not observed at 26 or 28°C. The proportion of larvae settled on the skeleton of dead animals was similar between the 26 and 30°C (7%) but was lower at 28°C (1%).

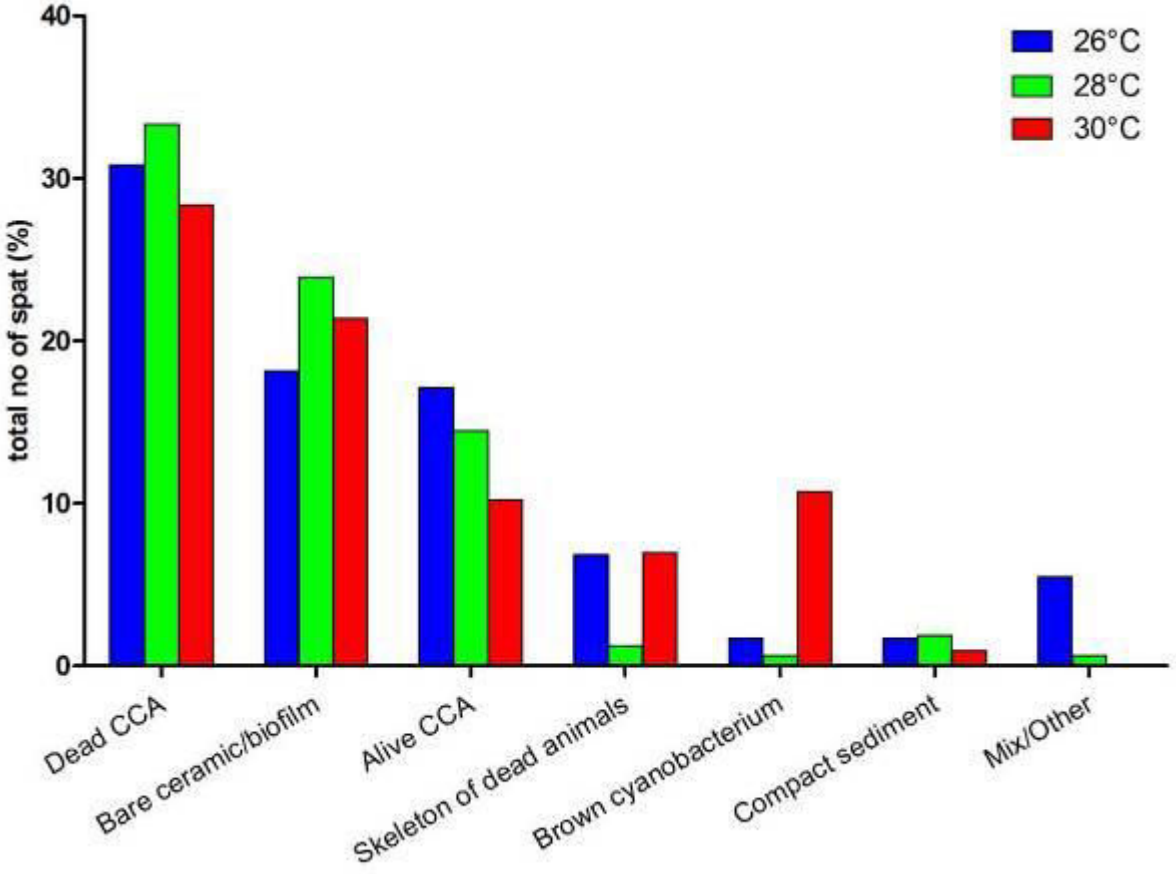


Figure 42: Substratum selection by *P. daedalea* recruits under the three temperatures treatments. CCA: Calcareous coralline algae. N=250, 184, 132 spats at 26, 28 and 30°C respectively

Most settlement was observed on the upper surface of tile under the three temperature regimes (Fig. 13), and no significant difference in the settlement position of spat on tiles (upper surface or edges) was found between the three temperature treatments (Main-effect ANOVA, $p > 0.05$). This trend was consistent between replicate and tile (main-effect ANOVAs, $p>0.05$)

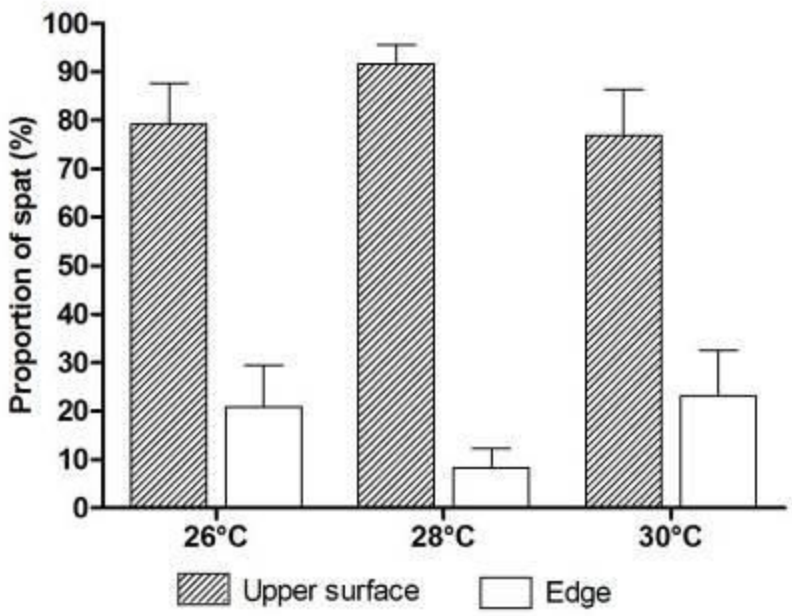


Figure 43: Settlement position of *P. daedalea* spats under the three temperature treatments. N =250, 184, 132 spats at 26, 28 and 30°C respectively

3. Juvenile development

3.1.Rate of development

Six stages of development were identified based on the comprehensive description made in part 1 (this chapter); they are summarised in Table 25. They were used to quantify the rates of juvenile coral development in each temperature treatment illustrated in Figure 14A.

Table 25: Stages of development in *P. daedalea* from settlement to juvenile polyp.

Code number	Development stage
0	Attachment
1	Metamorphosis
2	Primary polyp with 9 tentacles
3	Primary polyp with 12 tentacles
4	Primary polyp with 15 to 18 tentacles
5	Primary polyp with 24 tentacles or more

The breeding temperature had a significant influence on the development rate of coral spats (Main-effect ANOVA, $p<0.01$). The development rate of juvenile coral remained the slowest at 30°C and was significantly different compared to the those of the 26 and 28°C treatments (Fisher LSDs, 26 *vs.* 30°C, 28 *vs* 30°C, $p<0.01$). It was the fastest at 28°C during the two months after spawning, but became then similar as the control treatment. Overall no significant difference in the development rate of the juvenile corals was found between the 26 and 28°C treatments (Fisher LSDs, $p>0.05$). Apart from the temperature, the development rate of coral was found to vary between settlement tiles (Main-effect ANOVA, $p<0.001$) but not between replicates (Main-effect ANOVA, $p<0.001$). After five months of development, 57% of the coral spats reached the last development stage (Stage 5) at 26°C, while it was 24% at 28°C and 10% at 30°C.

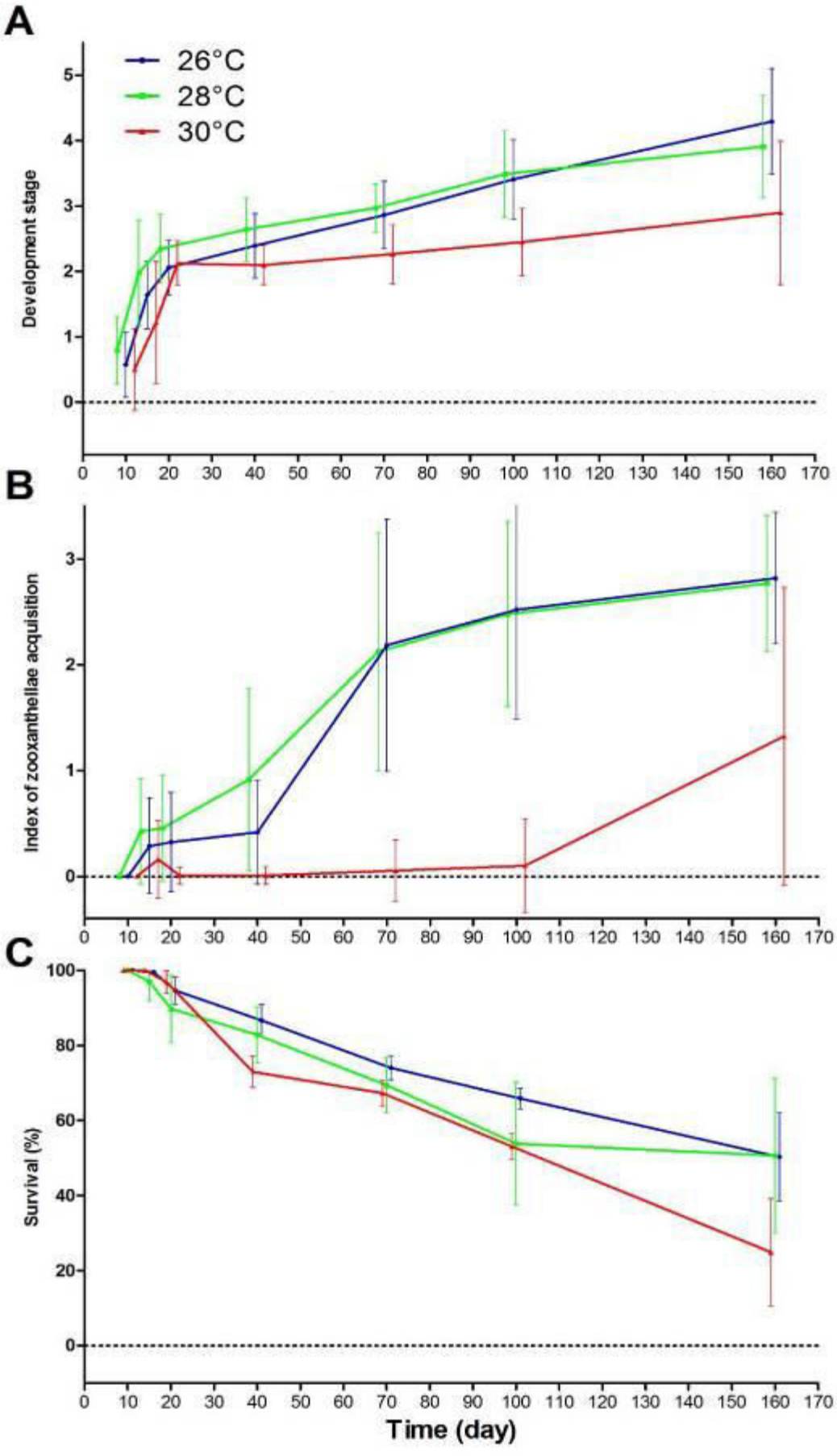


Figure 44: Juvenile development (A), acquisition of zooxanthellae (B) and mortality (C) in *P. daedalea* under three regimes of temperature.

3.2.Zooxanthellae acquisition and bleaching

The concentration of zooxanthellae in the tissue was estimated from the direct observation of the coral spats over time. Four levels of zooxanthellae infestation were defined and are illustrated in Figure 15. Level zero corresponded to the complete absence of zooxanthellae in the tissue. This level was observed on young or bleached recruits. In level 1, the zooxanthellae occupied 1-10 % of the total surface of coral tissue (Fig. 15 a). At this stage, they were sparse and it was still possible to distinguish them from each others. In level 2 and 3, the zooxanthellae occupied respectively 11-50% and more than 50% of the polyp tissue (Figs. 15 b and c).

The acquisition of zooxanthellae over time by the juvenile polyp is illustrated in Figure 14 B. It started simultaneously in the three temperature treatments at 14 DAS. The concentration of zooxanthellae in the polyp tissue increased then rapidly at 28°C and remained the highest compared to the two other treatments for the first two months of development. It became then similar between the 26 and 28°C until the end of the experiment. Overall, no significant difference was found in the concentration of zooxanthellae in the polyp tissue between the 26 and 28°C treatment (Fisher LSD, 26 *vs* 28°C, $p>0.05$). In contrast, the rate of zooxanthellae acquisition was significantly different in the warmest treatment compared to the two other treatment (Fisher LSDs, 26 *vs* 30°C and 28 *vs* 30°C, $p<0.001$). In this treatment, most polyp lack zooxanthellae or exhibited very low concentration of the algae in their tissues. In total, 68% of the surveyed colonies showed no zooxanthellae in their tissues for the duration of the experiment. Nevertheless, in other coral recruits maintained at 30°C, the concentration of zooxanthellae in the tissue increased slowly after two months of development (70 DAS) and then rapidly between three and five months. After five months of development, a total of 17 colonies (43%) over the 40 colonies alive in the three 30°C replicates contained a high concentration of zooxanthellae (level 2 or 3). This corresponded to 2, 4, and 11 recruits in replicate 1, 2 and 3 respectively.

Bleaching or the complete loss of zooxanthellae in the polyp tissue was observed in the two warmest treatments but not in the control treatment (Table 26). It was regular in recruit maintained at 28°C and concerned 1-5% of the coral at each sampling date (Table 26). At 30°C, a 12% bleaching was observed at 20 DAS. No subsequent bleaching was observed in this treatment.

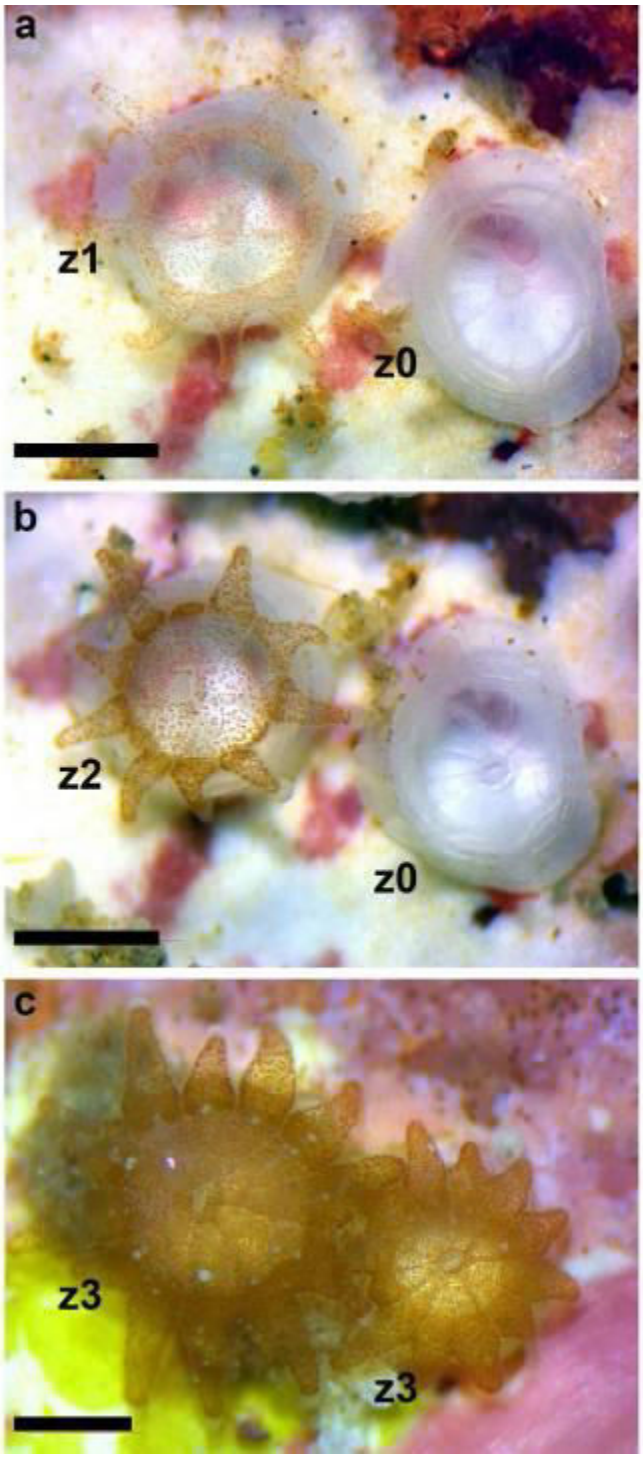


Figure 45: Zooxanthellae acquisition in *P. daedalea* recruits at a).15 days, b) 1 month, c) 2 months after spawning. z0-z3: index of zooxanthellae infestation in the polyp tissue. Scale bars are 500µm

Table 26: Proportion of bleached recruits (%) of *P. daedalea* under the three temperature treatments.

Days after spawning	T26	T28	T30
10	-	-	-
15	-	2.70	-
20	-	4.87	12.89
40	-	4.80	-
70	-	-	-
100	-	1.33	-
160	-	1.49	-

2.1.Survival

The survival of the juvenile coral over time is illustrated in Figure 14 C. The rate of mortality increased gradually over time in the three temperature treatments. It was higher at 28°C until 20 DAS compared to the two other treatments, it became then the highest at 30°C until the end of the experiment. On average, 75% of the recruits that had settled at 30°C died after five months. This mortality rate was significantly higher than in the 26 and 28° treatments (Fisher LSDs, 26 *vs* 30°C, $p<0.001$; 28 *vs* 30°C, $p<0.01$). In contrast, no significant difference was found in the mortality rate between the 26 and 28° treatments (Fisher LSD, $p>0.05$), that averaged 50% at the end of the experiment. Recruit mortality was nevertheless highly variable and was found to significantly vary between tiles and replicates (Main-effect ANOVAs, $p<0.05$).

Discussion

In this study, we provided a complete description of the larval development in *Acropora austera,* and the larval and juvenile development in *Platygyra daedalea* based on the observations of eggs obtained from the cross fertilisation of ten colonies and the settlement of more than 500 recruits. This was possible through the carefully design of an aquarium set-up that proved to be adequate to maintain the juvenile corals of *P. daedalea* for seven months after spawning.

1. Embryogenesis and larval development

This study provided the first detailed description of embryogenesis and larval development in *A. austera* and *P. daedalea.* The development of gamete into a planula larva in the two studied species was consistent with this reported in the literature for other Acroporidae and Faviidae occurring on more tropical reefs (Tables 27 and 28). The frequent samplings of embryos and larvae allowed the observation of intermediate development stages such as the "blastula donut" and "blastula prawn-chip" or the "planula pear-shape" and "the elongate planula". These intermediate developmental stages have been reported in other coral species such as *Platygyra contorta*, *Montipora hispida* (Okubo et al. 2013), *Acropora hyacynthus* (Hayashibara et al. 1997), *Acropora millepora* (Ball et al. 2002). Although non-official, their given names are now widely used in embryonic studies (Hayashibara et al. 1997; Ball et al. 2002; Okubo et al. 2013).

The observation of intermediate development stages during planulation was of particular importance as it indicated the planula competency. The early planulae of *A. austera* and *P. daedalea* (pear-shape stage) were swimming randomly and remained buoyant in the kreisels suggesting that they were not yet competent. In contrast, the elongate planulae were sinking toward the bottom of the kreisels and started attaching on the net. The difference in competency between the early and late planula stages was confirmed during a first trial to induce settlement in *P. daedalea* of South Africa at 3 DAS when most of the larvae were still at the pear-shape stage. No settlement was observed in the following three days suggesting that the larvae were not yet competent. In contrast, settlement on tiles was observed two days after the addition of 6 day-old planulae that had reached the elongate stage. The histological processing of the elongate planula in several coral species has revealed the presence of diverse cell types in the ectoderm (e.g. nematocysts, granular cells, Okubo et al. 2013), that

may that may play a role in the substratum selection and attachment of larva (Chia & Bickell 1978; Heyward 1987; Leitz 1997).

At the end of the larval development, some *s*even-day old planulae of *P. daedalea,* showed signs of early metamorphosis (four primary mesenteries) while they were still swimming in the kreisels. This primary transformation has also been observed in *Platygyra lamellina* planulae of the same age (Shlesinger & Loya 1991) and in several other coral species (Richmond 1985; Babcock & Heyward 1986). It may coincide with the absence of a suitable settlement surface in the experimental kreisels (Harrigan 1972; Richmond 1985; Harrison & Wallace 1990). In one occasion, the metamorphosed planulae of *P. daedalea* were observed attaching to each other with a mucus (at 12 DAS). The use of mucus for the attachment of larvae on a substratum has been reported in several studies on scleractinian corals (reviewed in Harrison & Wallace 1990; Okubo et al. 2013). Little is known about this process and we provided one of the first microphotographs that illustrates this process.

Table 27: Comparison of larval and juvenile development in selected *Acropora* spp in aquarium. Hyphen indicates no data.

	Acropora austera	*A. hyacynthus, A florida, A. nasuta, A.secale*	*A. tenuis*	*A. digitifera*	*A. millepora*
Location	South Africa (27°S)	Japan (26°N)	Japan (26°N)	Western Australia (23°S)	Eastern Australia (14°S)
Temperature at spawning	26°C	25-26°C	26-26.5°C	27-28°C*	28°C
First cleavage	2 h	2h	2 h	3 h	3 h
Morula	6 h	8 h	6 h	-	9 h
Blastula	10 h	12-17 h	7-10 h	10h	13 h
Gastrula	24 h	24-32 h	18-20 h	18 h	36 h
Planula	1.5-4 d	1.5-3 d	1.5-9 d	-	3-4 d
Settlement	7 d	6-21 d	-	6-8 d	-
First zooxanthellae acquisition	3 mo	9 d	-	-	-
Primary polyp	10 d	50 h after settlement	-	-	-
Complete skeleton print	12 d	6 d after settlement	-	-	-
Budding	1 mo	1 mo	-	-	-
Source	present study (ambient temperature)	Hayashibara *et al*. (1997)	Okubo and Motokawa (2007)	Gilmour (1999)	Ball *et al*. (2002)

Table 28: Comparison of larval and juvenile development in selected Faviidae. Dashed line indicates transfer of propagules to sea.

	Platygyra daedalea	*P. lamellina*	*P. sinensis*	*Favia favus*
Temperature at spawning	26°C	27°C[1]	27-28°C	27°C[1]
Location	South Africa (27°S)	Red Sea (29°N)	Great Brrier Reef (18-19°S)	Red Sea (29°N)
First cleavage	2h	2h	2h	2h
Morula	4-6 h	6-8 h	N/A	6-8 h
Blastula	10-15 h	14-16h	7-10 h	14-16h
Gastrula	18 h	20 h	26 h	20 h
Planula	1-5 d	2-4 d	1.5-2 d	2-4 d
First settlement	8 d	8-9 d	4-7 d	7 d
Initial zooxanthellae acquisition	14 d	16-18 d	13d	26-30 d
Primary polyp	24 d	20-24 d	10 d	26-30 d
Complete skeleton print	7 mo	3 mo	8 mo[2]	3 mo
Budding	7 mo	7-9 mo	N/A	7-9 mo
Source	present study (ambient temperature)	Shlesinger and Loya (1991)	Babcock and Heyward (1986)	Shlesinger and Loya (1991)

[1] Ben-David Zaslow *et al*. (1999)
[2] Babcock et al. (2003)

2. Influence of temperature on embryogenesis and larval development

The rate of embryogenesis and larval development in coral is known to be influenced by the seawater temperature. Aquarium assays have shown that slightly elevated temperature (2°C above ambient) induces a faster larval development and a diminished pre-competency period in several coral species (Nozawa & Harrison 2000; Edmunds et al. 2001; Nozawa & Harrison 2007; Randall & Szmant 2009b; Heyward & Negri 2010). In this study, the breeding temperature had different effects on the early life of the two studied species. In *A. austera,* no influence of temperature was noted during the embryogenesis (until the gastrula stage) but the larval development was accelerated at high temperature (28°C) and slowed down at lower temperature (24°C). This resulted in a three-day delay in the apparition of the competent planulae between the 24 and 28°C treatments. Similar responses to slightly elevated temperature during larval development have been observed in *Porites astreoides* (Edmunds et al. 2001) and in *A. millepora, A spathulata, Fungia repanda* and *Symphyllia recta,* that

showed shorter pre-competency period when exposed to 2°C above ambient temperature (Heyward & Negri 2010). O'Conner and co-authors (2007) demonstrated that increased temperature tend to increase metabolism which in return enhanced the larval development. In contrast, a lower temperature tend to reduce the rate of development as observed in this study when larvae of a. austera were exposed to 2°C below ambient (24°C) and in *F. repanda* at 2°C below ambient temperature (i.e. 26°C, Heyward & Negri 2010) and in *Goniastrea australensis* at 4°C below ambient temperature (i.e. 22°C, Wilson & Harrison 1997).

In *P. daedalea* off South Africa, the temperature had no significant effect on the rate of embryogenesis and larval development in the two heated treatments (2°C and 4°C above ambient, Table 29). No influence of temperature was also found on the larval development of other Faviidae corals such as *Favites abdita, F. chinensis* of Japan (26°N) exposed to temperature of 1-5°C above ambient (27°C, Negri et al. 2007), suggesting that the embryos and larvae of this family may be more resistant to thermal stress than in other coral families. Nevertheless, other studies on the influence of temperature on *Faviidae* coral reported conflicting results. Impaired development was observed in *Diploria strigosa* of the gulf of Mexico (27°N) when exposed to temperature of 1-3°C above ambient (Bassim et al. 2002; Bassim & Sammarco 2003). In addition, a shorter pre-competency period suggesting a faster larval development was observed in *P. daedalea* on the Great Barrier Reef (23°S) exposed to 2°C above ambient temperature (Nozawa & Harrison 2000). Coral may use various cellular mechanisms in response to temperature stress including heat shock proteins, glutathione, ubiquitin and superoxide dismutases (Brown et al. 2002; Baird et al. 2009a). Similar proteins have been observed in some coral larvae, including those of the Faviidae *Montastrea faveolata that* may explain their possible resistance to heat. (Meyer et al. 2009; Rodriguez-Lanetty et al. 2009; Voolstra et al. 2009; Polato et al. 2010). The thermal response of coral larvae is nevertheless variable and may be link to phenotypic plasticity or physiological acclimation to native habitat (Polato et al. 2010). For example, variation in gene expression of the coral larvae of *M. faveolata* in response to heat stress was observed between larvae collected from colonies of Mexico and Florida (Voolstra et al. 2009; Polato et al. 2010).

3. Survival and dispersal potential

The survival rate of *A austera* embryos and larvae was the lowest in the warmest treatment and the highest in the coolest treatment. Nevertheless, the high mortality of embryos and larvae of *A. austera* in the warmest treatment (28°C) was compensated by their fast

development rate. The survival rates in the warmest and control treatments were indeed similar at the end of the planula development (c.a. 35%). In contrast, only 7% of the larvae completed their development in the in the coolest treatment (24°C). Our data on the larval development of *A. austera* in South Africa, suggest that a slight increase in the seawater temperature following spawning (2°C above ambient) may favour the rapid settlement of larvae and their retention on the reefs. On the opposite, cooler temperature (2°C below ambient) may increase their dispersal but reduced their probability of survival.

In *P. daedalea*, although the temperature had no effect on the development of early-life stages, it affects the survival of the embryos and larvae. For the first four days after spawning (96h), survival was significantly lower in the heated treatments (28 and 30°C) than in the control (26°C). This period corresponded to the embryonic development, from the fertilised egg to the early planula. After 96h, survival in *P. daedalea* larvae became similar between the 26 and 28°C treatments but remained significantly lower at 30°C. In *Acropora palmata* (Randall & Szmant 2009b) and *D. strigosa* (Bassim & Sammarco 2003) exposed to elevated temperature (2-4°C above ambient), survival was also found to be lower during the embryonic stage than the larval development, suggesting that this phase may be particularly sensitive to temperature in certain species. In *P. daedalea*, the larval development ended at 8 DAS with the settlement of larvae and this occurred simultaneously in the three temperature treatments. Settlement occurred also simultaneously in *P. daedalea* larvae of the Great Barrier Reef exposed to ambient (27°C) and slightly elevated temperature (29°C, Nozawa & Harrison 2000), which indicates that slight increase in temperature may not affect the dispersal potential of larvae. Nevertheless, in *P. daedalea* of South Africa, new settlement was observed up to three months after spawning (100 DAS), while it stopped at 70 DAS in the heated treatments (28 and 30°C). The value found in the control treatment ranged within those reporied in *P. daedalea* on the Great Barrier reef (93-124 DAS, Nozawa & Harrison 2000)Temperature increase in the context of global warming may therefore affect the mesoscale dispersal of larvae in this species.

4. Settlement

In this study, settlement and subsequent juvenile development were observed in *P. daedalea* but not in *A. austera* for technical reasons. The larvae of *P. daedalea* of South Africa were observed searching the substratum for 2-3 days (6-8 DAS) before attachment. Metamorphosis started rapidly after attachment and a whitish primary polyp appeared on day later (9 DAS).

The primary polyp was fully formed 5 days later (14 DAS) showing extended tentacles and a basal ring. A similar timing of attachment and metamorphosis was observed in other Faviidae such as *P. lamellina* and *Favia favus* in the Red Sea (15 DAS, Shlesinger & Loya 1991) and in an Acroporidae *Acropora secale* in Japan (12 DAS, Hayashibara et al. 1997). At ambient temperature, 22-25% of the larvae introduced in each replicate settled on the preconditioned tiles. This settlement rate ranged within this reported (7-41%) in laboratory experiment on *P. daedalea* on the Great Barrier Reef (Nozawa & Harrison 2000; Miller & Mundy 2003). Settlement was significantly lower in the 28 (14%) and 30°C (13%). Similarly, a higher settlement rate was also found at ambient temperature than in the heated treatments in *Acanthastrea lordhowensis, G. australensis* (Wilson & Harrison 1997), *D. strigosa* (Bassim & Sammarco 2003), *A. palmata* (Randall & Szmant 2009b) and *Favia fragrum* (Randall & Szmant 2009a) suggesting that this phase is highly sensitive to increase in temperature. Most studies have focused on the larval development of coral to evaluate the influence of increasing temperature on reef recovery and replenishment by new coral propagules (see for example Edmunds et al. 2001; Baird et al. 2006; Krupp et al. 2006; Negri et al. 2007), nevertheless the study of coral settlement under different temperature regimes may reveal different response of coral to temperature.

5. Substratum selection

In this study, most larvae of *P. daedalea* (49% of spats) settled on the ceramic tiles or on the top of the dead calcareous coralline algae. These substrata may have been covered by a microbial biofilm that could have attracted the coral recruits for settlement. Live calcareous algae (*Mesophyllum* sp) were the third preferred substratum (after dead coralline algae and bare ceramic) and collected 17% of the recruit. Calcareous coralline algae are known to produce chemical signals which induce metamorphosis and settlement by many coral larvae (Johnson et al. 1991; Negri et al. 2001; Golbuu & Richmond 2007; Ritson-Williams et al. 2009; Gleason & Hofmann 2011). There are, however, few reports of coral larvae settling preferentially on a biofilm and none of these concern Faviidae (Negri et al. 2001; Webster et al. 2004; Petersen et al. 2005; Golbuu & Richmond 2007). For example, the faviid *Goniastrea retiformis* settled preferentially on the CCA *Hydrolithon reinboldii* rather than on a microbial biofilm in the laboratory (Golbuu & Richmond 2007).

The temperature had no significant influence on the choice of substratum for settlement in *P. daedalea* larvae of South Africa that was highly consistent between treatments. In addition,

no significant difference in the position of coral spats on tile was observed between the three temperature treatments and most spats settled on the upper surface of the tiles. The lack of difference in settlement preference in *P. daedalea* between the temperature treatments may indicate that the choice of substratum for settlement is intrinsic to the species and does not vary with temperature. In contrast, Putnam and co-authors (2008) found that the larvae preference for CCA increased between 23°C and 29°C (ambient 25°C) to the detriment of limestone in the brooding coral *Stylophora pistillata* (Putnam et al. 2008). They suggested that the change in larval settlement preference may have been due to the increased potency of the morphogen contained in the CCA with increasing temperature, or to the reduced swimming activity of larvae at high temperature that may have resulted in a fast and indiscriminate settlement (Putnam et al. 2008). In *P. daedalea* of the Great Barrier Reef, the settlement orientation of recruits did not vary between temperature treatments but among settlement runs carried out at 9, 21 and 69 days after spawning (Nozawa & Harrison 2000). This may have been the results of a reduction in buoyancy and motility of larvae at the end of the pre-competency period (Nozawa & Harrison 2000). These conflicting results provide a strong incentive to further investigate the substratum discrimination, motility and settlement behaviour of larvae under different regimes of temperature.

6. Juvenile development

The development from metamorphosis to juvenile polyp in *P. daedalea* at ambient temperature was consistent with the timing of development in other Faviidae such as *P. lamellina* and *F. favus* of Red Sea (Shlesinger & Loya 1991) and *P. sinensis* of the Great Barrier Reef (Babcock & Heyward 1986, Table 8, appendix A). Nevertheless, polyp growth was slightly slower in *P. daedalea* raised in aquaria in South Africa than in *P. lamellina* raised in the Red Sea (Shlesinger & Loya 1991). This may be due to species-specific characteristics or to the laboratory conditions; the use of artificial light may not have been as effective as natural light for photosynthesis and therefore diminished the growth rate. Moreover the polyps were not fed in this experiment, whereas the *P. lamellina* recruits would have had natural prey in the Red Sea. Feeding has been observed in juvenile polyps from two days after settlement and is believed to increase survival (Toh et al. 2013).

The concentration of the symbiotic zooxanthellae in *P. daedalea* polyp of South Africa remained low (level 1 or 2) for the first two months of development. The zooxanthellae are known to provide up to 95% of the carbon requirements of the animal partner (Muscatine

1990). As larval metamorphosis and early development may be energy-consuming processes, the development rate of the *P. daedalea* recruits was expected to vary depending on the zooxanthellae concentration in the polyp tissue. Juvenile development in *P. daedalea* was nevertheless gradual suggesting that the primary polyp may benefit from another source of energy in the absence of zooxanthellae. In *Acropora tenuis*, some larvae that settled rapidly still contained a high quantity of lipid, that could have serve as a metabolic substrate for settlement (Harii et al. 2007). In addition, the discovery of symbiotic nitrogen-fixing cyanobacteria in the tissue *Montastrea cavernosa* suggests that coral symbiont other than the zooxanthellae may supply the host with essential elements such as nitrogen to meet its energy requirement (Lesser et al. 2007). The source of energy and energy requirement during juvenile polyp development are presently unstudied. This stage of development provides nevertheless a good opportunity to understand the global energetic in coral as it exhibits both the aposymbiotic and symbiotic stages.

During the first month of development, the skeletal print in *P. daedalea* was devoid of septa and limited to the epitheca. The formation of septa started two months after spawning and the skeleton print was complete and fully recognisable seven months after spawning (see description in Babcock et al. 2003). The slow calcification observed in this study was consistent with that reported for *P. daedalea* (Babcock et al. 2003) and *P. sinensis* on the Great Barrier Reef (Babcock & Heyward 1986), raised respectively in aquaria and at sea (Table 2). The formation of septa was reported earlier in *P. lamellina* and *F. favus* in the Red Sea, i.e. three months after spawning (Shlesinger & Loya 1991). Overall, the slow skeletal development observed in Faviidae may explain why these corals are rarely encountered on artificial settlement tiles usually immersed for three months on reefs (see for example Fisk & Harriott 1990; Hughes et al. 2002; Adjeroud et al. 2007; Mangubhai et al. 2007). Indeed, the identification of early skeleton prints of corals is difficult and can even be confused with those of barnacles (pers obs). The detailed description of early calcification in *P. daedalea* provided in this study completes the description by Babcock et al. (2003) and will help to identify incomplete skeleton prints of this genus on settlement tiles.

7. Influence of temperature on juvenile development

High temperature (4°C above ambient) significantly affected the rate of development, the concentration of zooxanthellae and the survival in juvenile polyp of *P. daedalea* in South Africa (Table 29). In contrast, there was no significant difference in these parameters between

the control and the slightly heated treatments (2°C above ambient). These results suggest that the upper thermal limit of *P. daedalea* juveniles in South Africa is close to 30°C; nevertheless, they may tolerate a slight increase in sea temperature by 2°C. To our knowledge, no other study has investigated the influence of temperature on juvenile development for further comparison with this work.

The influence of high temperature on *P. daedalea* juvenile polyp was the most evident after 20-30 days. Before this time, normal metamorphosis and early-development (up to the primary polyp with 9 tentacles) were observed in most larvae exposed to 30°C. In addition, the mortality of corals at 30°C ranged within this of the control and slightly heated treatments. The zooxanthellae acquisition in juvenile polyp exposed to 30°C was simultaneously as in the two other temperature treatments, yet the concentration of zooxanthellae in the polyp tissue remained lower than at 26 and 28°C. A 12 % bleaching was observed at 20 days in the juvenile polyps exposed to 30°C. After it, most recruit of the warmest treatment remained completely bleached for the rest of the experiment and showed halted development and low survival. Nevertheless, a sudden increase in the number of polyp containing zooxanthellae (5%) was observed at three months after spawning and continued until five months (51%). It was accompanied by a fast polyp development. At the end of the experiment, 5 recruits of the warmest treatments reached an equivalent stage of development as those observed in the control treatment and a further 6 recruits were at the development stage 2 or 3. This unusual pattern concerned a very limited number of recruit (17 over the 166 that settled in this treatment). Nevertheless, it suggested that some *P. daedalea* recruits may have acclimatise to the warmest temperature conditions.

Two hypotheses may explain the survival and development of these recruits in the warmest temperature treatments while most of the other did not survived under this condition. First, the crossing of 10 coral colonies may have provided genetic variation in the thermal physiology of recruits, and some genetic combinations may have shown greater resistance to high temperature than other. Substantial genetic variance in response to temperature has been observed in *A. millepora* larvae exposed to 32°C, in particular in the expression of a stress gene (βγ-crystallin) that has fitness consequences (Meyer et al. 2009). Secondly, some clades of zooxanthellae such as the clade D are known to be more resistant than other to heat stress (Rowan 2004; Berkelmans & van Oppen 2006; Ladner et al. 2012). The acquisition of zooxanthellae of a resistant clade may have provided the primary polyp with sufficient energy

to continue its development at 30°C. Unfortunately, no genetic study or identification of zooxanthellae was made in this study to verify these hypotheses. This result nevertheless suggests that there is potential mechanism for *P. daedalea* juveniles to acclimatize to high temperature.

8. Summary and conclusion

In summary, we found that the larval and juvenile development in the subtropical corals *A. austera* and *P. daedalea* was similar with this reported in the literature for other Acroporidae and Faviidae occurring on more tropical reefs. Larvae of *A. austera* were more sensitive to heat stress than those of *P. daedalea* and a slight increase or decrease in temperature strongly influenced the rate of larval development and mortality in this species. The negative effect of heat stress were visible from 28°C (2°C above ambient) in *A. austera* larvae, which is the temperature at which the adult colonies start bleaching (Celliers & Schleyer 2002). This species is however found on tropical and equatorial reefs (e.g. French Polynesia or Singapore, Veron 2000), where the mean summer temperature often reaches 30°C (Babcock et al. 1986; Carroll et al. 2006). In South Africa, *A. austera* may have acclimatised to the local temperature regime, where the summer maxima rarely go above 28°C (ORI 1994-2012, unpublished data). In the context of global warming, the recruitment success of this species is of concern in South Africa, as the seawater temperature may rapidly approached the local upper thermal limit for larvae development and survival.

A different scenario may be observed in *P. daedalea* of South Africa. Its larvae and subsequent primary polyps were not affected by the slightly heated treatment (28°C) although a lower number of coral settled in this treatment. Settlement in *P. daedalea* was late in the three temperature treatments (15 days after spawning) compare to the usual period of coral settlement (4-6 days after spawning, Harrison & Wallace 1990) suggesting a potential for large-scale dispersal. The high temperature (30°C) had a drastic effect on the development, survival and zooxanthellae acquisition of the vast majority of the settled larvae suggesting that this temperature may represent the upper thermal limit for this coral in South Africa. As in *A. austera*, this limit was lower than this reported in other *Platygyra* spp on tropical and equatorial reefs (Veron 2000) and suggested some degree of temperature acclimation to the local condition on the reef. In the warmest treatment, most recruit of *P. daedalea* died after five months of experiment but 17 of the 166 that had settled, showed the sudden acquisition

of zooxanthellae and increased development rate from three to five months after spawning. This suggested that they may have acclimatised to the high temperature.

Subtropical and high-latitude reefs may provide refuge against high temperature in a first time as they are generally exposed to lower temperature regime than in the tropics (Riegl & Piller 2003; Greenstein 2008). Nevertheless, the coral communities on these reefs have acclimatised to the local temperature conditions and their upper limit of thermal tolerance had become lower than in their counterparts occurring in the tropics. The coral communities may therefore be exposed to a higher risk of extinction with the predicted increase of temperature as their upper thermal limit will be reached rapidly. Nevertheless the genetic mixing ensure by sexual reproduction (Lesser et al. 2007) or the change in symbiont partner (Ladner et al. 2012) may be potential mechanism for corals to acclimatize to warming oceans.

Table 29: Summary of the influence of temperature o the early life stages of *P. daedalea*

		Ambient (26°C)	**28°C**	**30°C**
Larval phase	Rate of development	Same rate between treatments		
	Survival	No difference		Lowest rate
Settlement	Total number of settled coral	Highest number	Intermediate	Lower
	Peak of settlement	Earlier at 28°C until 10 d then similar to the other treatments		
	Substratum preference	Same (dead calcareous coralline algae)		
	Position	Same (upper surface of tiles)		
Juvenile development	Development rate	Similar rate		Lowest rate
	Zooxanthellae acquisition	Simultaneous		
	Concentration of zooxanthellae	Similar concentrations over time		Lowest concentration
	Mortality	Similar rate over time		Lowest rate
	Survival	Similar rate over time		Lowest rate

Appendix A

Table 30: Main results of selected studies on the influence of temperature on the early life stages in corals. The studies are organised by date of publication. B: brooder, S: spawner.

Species	Location	Brooder/ Spawner	Temperature treatment	Aspect studied	Exposure	Main results	Source
Acanthastrea lordhowensis	Solitary Islands (30°S)	S	24,26,28,30°C	Larval development and settlement	7 d	Higher settlement at 26°C but not significantly different than in other temperatures treatments	Wilson and Harrison (1997)
Goniastrea australensis			22,24,26, 28°C			Slower larval development at lower temperature. Significantly higher number of deformed larvae in the heated treatments. Significantly more settlement at 26°C.	
Platygyra daedalea	Great Barrier Reef (Heron Island)	S	27,29°C Ambient: 27°C	Larval development and settlement	14 d	Reduced pre-competency period at higher temperature. Similar settlement and mortality rate in the control and heated treatments	Nozawa and Harrison (2000)
Porites astreoides	Florida (24°N)	B	26, 28, 33°C Ambient: 28°C	Larval development	24 h	High temperature increases larval mortality, and induces faster development. Larval motility not affected by high or low temperature	Edmunds *et al*. (2001)
Diploria strigosa	Gulf of Mexico (27°N)	S	30, 31, 32°C Ambient: 29°C	Embryogenesis	24 h	Developmental aberrations at high temperatures	Bassim *et al*.(2002)
			28, 30, 32°C Ambient: 29°C	Larval development	9 d	At high temperature, high mortality, slow development, reduced motility and settlement rate	Bassim and Sammarco (2003)
Acropora muricata	Okinawa (32°N)	S	28, 32, 36°C Ambient: 26-28°C	Larval development	7 d	All larvae died at 36°C. Equal survivorship at 28 and 32°C. No difference in survivorship between symbiont and asymbiontic larvae	Baird *et al*.(2006)
Fungia scutaria	Hawaii (21°N)	S	27, 29, 32, 33°C Ambient: 29°C	Embryogenesis, and larval developement	12 h	High survival at 27 and 29°C. Reproductive failure at 33°C	Krupp *et al*. (2006)

Table 1 (continued)

Species	Location	Brooder/ Spawner	Temperature treatment	Aspect studied	Exposure	Main results	Source
Acropora millepora, *Favites abdita,* *Favites chinesis* *Mycedium elephantotus.*	Okinawa (26°N)	S	26, 28, 30, 32°C Ambient: 27°C	Fertilisation and first cleavage	4h	Normal fertilisation and embryogenesis in *Favites* and *Mycedium* sp up to 32°C Inhibited fertilisation and development aberration in *A. Millepora*	Negri et al. (2007)
Acropora solitaryensis	Lord Howe Island (33°S)	S	20,23,26,29°C Ambient: 23°C	Settlement and post-settlement mortality	5 d	Greater settlement but higher post-mortality at higher temperature	Nozawa and Harrison (2007)
F. chinensis	Okinawa (26°N)	S	27, 31, 34°C Ambient: 27°C	Settlement and post-settlement mortality	1h	Greater settlement and lower post-settlement mortality after short term exposure to high temperature	
Favia fragum	Curacao (?°N)	B	28, 29, 31°C Ambient: 28°C	Larval development and settlement	48-191h	Short term exposure has no effect on larval survival. Lower survival during settlement than larval development at 31°C after 156 h of temperature exposure	Randall and Szmant (2009a)
Acropora palmata	Mexico and Florida (?°N)	S	28, 30, 31.5°C Ambient: 28°C	Embryogenesis, larval development and settlement	6-7 d	Higher temperature increase development rate of embryos, developmental abnormalities in the warmest treatment. Peaked mortality during gastrulation. Lower settlement rate at higher temperature	Randall and Szmant (2009b)
Fungia repanda	Okinawa (32°N)	S	26, 28, 30°C, Ambient: 28°C	Metamorphosis	12h	High temperature diminished pre-competency period. Delayed metamorphosis at lower temperature	Heyward and Negri (2010)
A. millepora *Acropora spathulata* *Symphyllia recta*	Great Barrier Reef (18°N)	S	28, 30, 32°C Ambient: 28°C	Larval development, settlement and metamorphosis	4d	High temperature diminished pre-competency period. Threshold at 32°C	

Appendix B

Table 31: Summary of the Logrank Mantel-Cox analyses for the comparison of the Kaplan-Meier survival curves in *P. daedalea* under the three temperature treatments.

All	**All treatments**	**26 *vs* 28**	**26 *vs* 30**	**28 *vs* 30**
Chi square	81,51	11,22	172,9	36,10
df	2	1	1	1
p value	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Significance	***	***	***	***

0-48h	**All treatments**	**26 *vs* 28**	**26 *vs* 30**	**28 *vs* 30**
Chi square	22,22	27,64	33,60	0,008832
df	2	1	1	1
p value	p<0.0001	p<0.0001	p<0.0001	0,9251
Significance	***	***	***	ns

48-96h	**All treatments**	**26 *vs* 28**	**26 *vs* 30**	**28 *vs* 30**
Chi square	16,84	12,92	59,04	4,039
df	2	1	1	1
p value	0,0002	0,0003	< 0,0001	0,0445
Significance	***	***	***	*

96h-228h	**All treatments**	**26 *vs* 28**	**26 *vs* 30**	**28 *vs* 30**
Chi square	12,91	0,1609	14,95	13,95
df	2	1	1	1
p value	0,0016	0,6883	0,0001	0,0002
p value summary	**	ns	***	***

Chapter III

Synthesis and general discussion

1. Main findings

Principal finding showed that the gamete development and breeding seasonality were similar in the two targeted species of South Africa and Reunion, despite contrasting environmental conditions between the reefs. The polyp fecundity and the recruitment rate were higher in South Africa than in Reunion while the opposite trend was expected. Pocilloporiade were the dominant spats on the settlement tiles at the two study sites. Acroporidae were the second most abundant spats in South Africa while it was Poritidae in Reunion and this pattern may reflect the trend observed in the adult communities. The influence of elevated temperatures on the early-life stages of coral was tested in aquarium experiment conducted in South Africa. *A. austera* appeared to be more sensitive to temperature increase in temperature than *P. daedalea*. These results will be discussed in the context of global changes and reef degradation.

2. Coral reproduction in a subtropical and a tropical reef

2.1. Reproductive traits and breeding seasonality

Marginal reefs, that lie at the limit of the coral distribution (Kleypas et al. 1999), have been the focus of recent studies as they may act as a refuge against high temperature and therefore promote coral survival in the context of global warming (Glynn 1996; Done 1999; Riegl 2003; Greenstein 2008). Nevertheless, the unusual conditions on marginal reefs have shown to interfere with coral reproduction and recruitment (Hayashibara et al. 1993; Harriott & Banks 1995; Harriott & Simpson 1997; Wilson & Harrison 1997; Hughes et al. 2002; Wilson & Harrison 2003; Nozawa et al. 2006; Nakamura & Sakai 2010). Difference in the breeding seasonality, time of spawning, and fecundity were therefore expected between the corals of South Africa and Reunion.

Our results showed that the breeding seasonality (September to February) and duration of gamete development (5-7 months) in *Acopora austera* and *Platygyra daedalea* were similar between South Africa and Reunion despite small variations (± one month) between years and study sites. These results suggest that the local conditions associated with this subtropical reef did not affect gamete development and spawning in the two study species. Active sexual reproduction and spawning has also been reported in two other scleractinian species, *Pocillopora verrucosa* (Kruger & Schleyer 1998) and *Hydnophora exesa* (Hart pers. com,

pers. obs) in South Africa proving that the coral reproductive capacity is maintained on these marginal reefs.

2.2.Fecundity

A higher fecundity index (size x number of oocytes per polyp) was found in South Africa than in Reunion in the two studied species. It resulted from a higher number of oocytes per polyp in the corals of South Africa compare to Reunion whereas little variation in the oocyte sizes was noted. *A. austera* off South Africa produced on average 43% more oocytes than its counterpart in Reunion and *P. daedalea* off South Africa contained twice the number of oocytes compared to Reunion.

These results suggested that corals in South Africa investigated a high effort into sexual reproduction and such strategy may be linked to the more stressful environmental conditions at sub-tropical latitude. High fecundity is commonly considered an adaptation to ensure the maintenance of a population in a complex environment when the stress is high (Price 1974; Williams 1975; Grime 1977; Hall & Hughes 1996; Maltby 1999). Active sexual reproduction and high fecundity was also observed in the high-latitude reefs of Western Australia (Wilson & Harrison 1997). Further comparison of the reproductive efforts in several coral species are nevertheless required to verify this trend on the marginal reefs.

In contrast, lower polyp fecundity was noted in Reunion which may indicate that more energy is allocated to other life functions than sexual reproduction (e.g. growth) on the stable tropical reefs. Low fecundity may also be the result of adverse environmental conditions that affect the reproductive output of the coral colonies (Rinkevich & Loya 1977; Richmond 1993; Ward & Harrison 2000). The Reunion reefs are submitted to intense anthropogenic pressures associated with coastal development, overfishing, and water pollution (Cuet et al. 1988; Naim et al. 2000; Tessier et al. 2008; Cuet et al. 2011). Nutrient enrichment is known to affect the fitness of the adult coral colonies as it intensifies the competition with macroalgae (Cuet et al. 1988; Littler et al. 2006), increases the water turbidity due to sediment load in the water (reviewed in Fabricius 2005), and impaired the coral physiology (e.g. calcification and photosynthesis, Ferrier-Pagès et al. 2000). In addition, coral fecundity is affected by increaseed concentration in nitrogen associated with urban and agricultural pollutions as shown in Ward & Harrison (2000) and Loya and co-authors (2004).

2.3. Spawning

Spawning in corals is likely to be dictated by a combination of environmental factors that ensure the synchronisation of gamete release between colonies and a high rate of fertilisation (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Oliver et al. 1988). Seawater temperature, solar radiation, and lunar phase are considered proximate cues, which operate at successively finer time scales (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Oliver et al. 1988; Penland et al. 2004). Since the seasonal variation in these parameters showed a different pattern between the tropical reefs of Reunion and the subtropical reefs of South Africa, the corals were expected to adapt their breeding seasonality and spawning period according to the local environmental conditions.

In South Africa, the mean average seawater temperature was 2°C lower than in Reunion. In addition, the rise in seawater temperature (August) and the annual temperature maxima (January) occurred one month earlier than in Reunion (Fig.1). The delay in seasonal increase of temperature had no influence on the onset of gametogenesis that was initiated in September-October in *A. austera* and *P. daedalea* at the two study sites. The increase in gamete size was strongly correlated with the increase in temperature (r^2= 0.64-0.92, chapter I, §7.1) in the two studied species from both localities. Nevertheless, no clear relationship was observed between the time of spawning and the annual peak in temperature maxima. Indeed, this peak was observed either during or up to two months after the end of gametogenesis (Fig.1). The small difference in the seasonal variation of temperature may explain why no major difference was observed in the breeding season and spawning period of the two studied species between South Africa and Reunion.

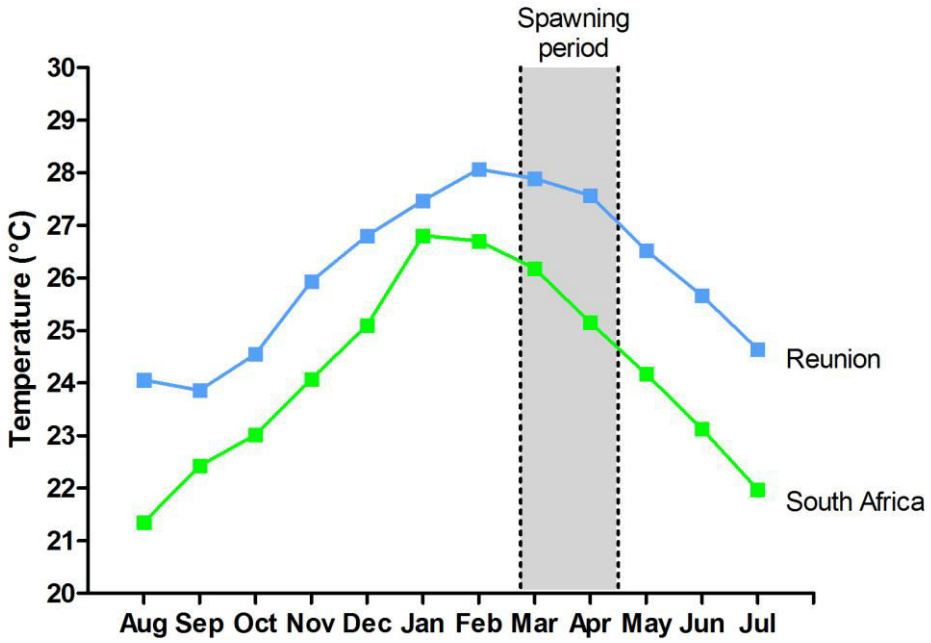
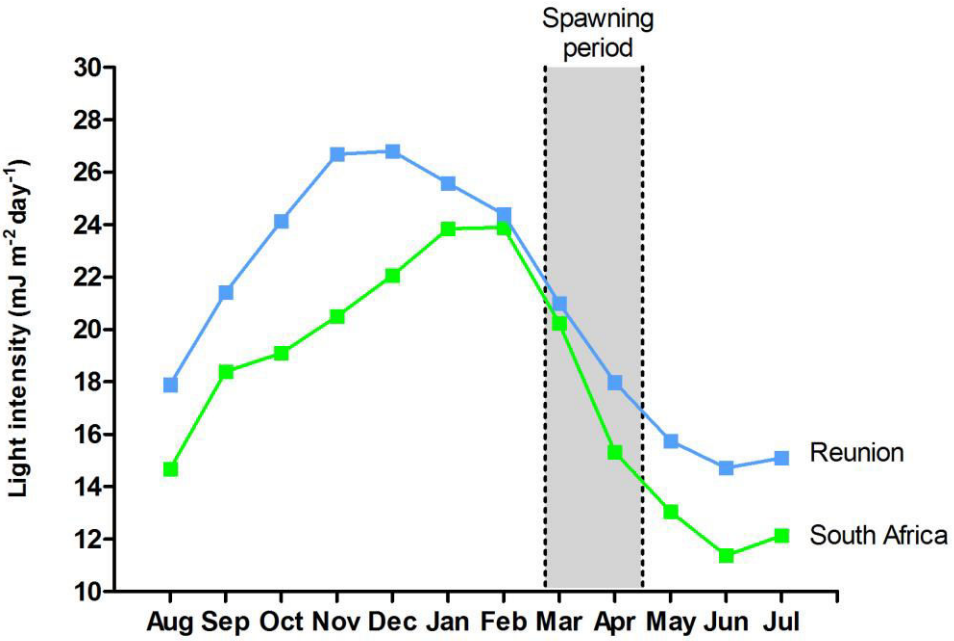


Figure 46: Mean monthly sea temperature (2010-2012) and spawning period in *A. austera* and *P. daedalea* in South Africa and Reunion.

The role of light intensity or rainfall in the synchronisation of gamete development and spawning of the two study species was less evident than for the temperature. In South Africa, a positive correlation was found between oocyte size and light intensity in *Acropora austera* but not in *P. daedalea*. Spawning occurred nevertheless following the summer peak in light intensity over the two year of study (Fig. 2). A combination of seawater temperature and light intensity may therefore control the timing of the breeding season and spawning in the subtropical reefs of South Africa. Light intensity has also proven to control gamete development or spawning in the Western Pacific (Penland et al. 2004) and the Caribbean (van Woesik et al. 2006).



] **Figure 47**: Mean monthly light intensity (2010-2012) and spawning period in *A. austera* and *P. daedalea* in South Africa and Reunion.

i

two year studied. These differencent responses to environmental factors suggest that the coral may have adapted to t local environmental conditions occurring on the reefs of South Africa and Reunion. Few studies have investigated rainfall as a trigger for coral reproduction (but see Mendes & Woodley 2002b). A meta-analysis at 19 sites worldwide has shown that coral spawning often occurred before or after the peak in heavy rainfall and in some case rainfall may be a better predictor for the timing of spawning than temperature (Mendes & Woodley 2002b). Heavy rainfall may diminish the rate of fertilisation and larval survival because of low salinity (Richmond 1996) and subsequent terrestrial run-off (see review in Fabricius 2005). In Reunion, large intrusion of surface fresh waters are observed on the reef during the rainy season (December to March, Cuet et al. 1988; Office de l'eau Réunion 2014, Joint pers com) and this may serve as a trigger for coral spawning.

Overall, the similarities in the reproductive mode and spawning seasonality of corals between South Arica and Reunion suggest that coral at subtropical latitudes may exhibit a reproductive pattern inherited from parent colonies on tropical reefs (Babcock et al. 1994). A similar case-study was reported on the high-latitude reefs of the Houtman Albrohos Atoll. The breeding season and timing of spawning in several coral species of the Houtman Albrohos Atoll was similar those of the tropical (Ningaloo) reefs in Western Australia, despite there being more

than two months difference in timing of the seasonal temperature minima between the two regions (Babcock et al. 1994). Babcock et al. (1994) suggested that most of the coral on the high-latitude reefs may have come from larvae transported by the poleward oceanic currents following mass spawning. In South Africa, the southward flowing Agulhas current carried the warm waters from the Mozambican channel and Madagascar (Lutjeharms 2006) and may have episodically transported coral larvae. This seemed to be corroborate by genetic studies that has shown evolutionary connectivity between coral populations of the south Western Indian Ocean (Macdonald et al. 2011; Montoya-Maya 2013).

3. Coral recruitment in a subtropical and a tropical reefs

3.1. Recruitment rate

The recruitment rate was higher in South Africa (548 ± 35 recruits m^{-2} per $year^{-1}$) than in Reunion (305 ± 27 recruits m^{-2} per $year^{-1}$). It was also higher than on the high-latitude reefs or eastern Australia (30°S, 132 recruit m-2 year-1, Harriott & Banks 1995) or western Australia (29°S, 17 recruits m-2 year-1, Harriott & Simpson 1997), but remained lower than the maximum rate reported on the Great Barrier Reef (15°S, 2044 recruit m-2 year-1, Fisk & Harriott 1990). The high recruitment rate in South Africa compared to Reunion did not verified the hypothesis that recruitment rate decreases with increasing latitudes (Harriott & Banks 1995; Harriott & Simpson 1997; Hughes et al. 2002; Nakamura & Sakai 2010).

Such a high recruitment in South Africa may be due to an important larval supply on the reefs (Fisk & Harriott 1990). As discussed in §2.3, the larval supply may come from the Madagascar and the Mozambican reefs with the Agulhas current (Lutjeharms 2006). Nevertheless, this scenario is unlikely as recent genetic studies have shown discontinuity between the coral populations of South Africa and Mozambique (Ridgway et al. 2001; Macdonald et al. 2011; Montoya-Maya 2013). An alternative explanation for this high recruitment rate may be the local entrapment of larvae due to reef-scale hydrodynamics (Morris 2009; Montoya-Maya 2013). Local wind-driven current at the time of coral spawning tend to promote the retention of larvae on reefs. In addition the high coral fecundity reported in the two studies species (Chapter 1) and other corals in South Africa (Kruger & Schleyer 1998, Hart pers com) may have contributed to the high level of local larval production. The retention and high production of larvae on the reefs may have contributed to the high rate of settlement observed in South Africa.

In Reunion, the recruitment rate (305 ± 27 recruits m^{-2} per $year^{-1}$) was lower than the values found in French Polynesia (17°S, 131 recruits m^{-2} $year^{-1}$, Gleason 1996) or Taiwan (24°S, 0-133 recruits m-2 year-1, Soong et al. 2003) but was much lower than at other tropical locations such as Japan (23°S, Nakamura & Sakai 2010) or the Great Barrier Reef (15°S, 2044 recruits m-2 year-1, Fisk & Harriott 1990) and in South Africa (see above). Several explanations exist for the low rate of recruitment recorded in Reunion. The first is that the reefs of Reunion are isolated from potential sources of larvae in the Indian Ocean. The island is washed by the South Equatorial Current (SEC) that crosses the Indian Ocean for 6500 km before reaching the Mascarene Plateau (Schott et al. 2009). The modelling of the surface currents between the nearby island of Mauritius and Reunion has shown a possible supply of larvae from Mauritius under certain environmental conditions (Crochelet et al. 2013). The Mauritian reefs are nevertheless limited in size and such larval supply may be negligible. The model developed by Crochelet and co-authors (2013) furthermore predicts that larvae spawned in Reunion may be carried away from the island to southern latitudes, thereby limiting self-recruitment on the reefs. In addition, the level of disturbance on the Reunion reefs may have affect the larval supply and recruitment rate on tiles (Richmond 1993; Connell et al. 1997; Loya et al. 2004; Fabricius 2005). As indicated in the paragraph 2.2, coral fecundity may diminish in disturbed areas and therefore limit the larval supply on the reefs (Ward & Harrison 2000; Loya et al. 2004). Low polyp fecundity was observed in *A. austera* and *P. daedalea* off Reunion compared to their South African counterparts (paragraph 2.2). Moreover, nutrient enrichment caused by the pollution of coastal water induces the development of fleshy and turf algae that compete for space and light with corals (Cuet et al. 1988; Littler et al. 2006; Naim 2006) and may strongly limit coral settlement (Tomascik 1991; McCook 2001; Birrell et al. 2005). Sedimentation due to coastal erosion may also prevent the larval settlement (Hunte & Wittenberg 1992; Birrell et al. 2005). The combinations of relative reef isolation, low coral fecundity and impaired settlement may have therefore contributed to the low rate of recruitment observed in Reunion.

3.2. Family composition of recruits

Pocilloporidae were the dominant spat at the two localities (62 and 73% respectively in South Africa and Reunion). Acroporidae were the second most dominant in South Africa (28%), was and the Poritidae (18%) in Reunion. The taxonomic composition of the coral recruitment in South Africa was consistent with previous studies on coral recruitment made at this locality

(Glassom et al. 2006; Hart 2012). It was however unique compared to other high-latitude reefs that had a lower proportion of Acroporidae (~12%) and a higher proportion of Poritidae (~15%, values averaged from Harriott 1992; Harriott & Banks 1995).

In Reunion, the proportion of Acroporidae (4%) on the tiles was negligible compared to the Great Barrier Reef (Wallace 1985c; Fisk & Harriott 1990) and Japan (Nakamura & Sakai 2010) where Acroporidae are by far the most abundant spat on settlement tiles. Nevertheless, it was similar to the reefs in French Polynesia (Gleason 1996; Adjeroud et al. 2007), Taiwan (Soong et al. 2003) and Kenya (Mangubhai et al. 2007) that are characterised by strong anthropogenic pressures and commensurately low rates of acroporid settlement. Since Acroporidae are sensitive to perturbation at both the juvenile (Ward & Harrison 1997; Negri et al. 2007; Nozawa & Harrison 2007) and adult stage (Ward & Harrison 2000; Loya et al. 2001; Celliers & Schleyer 2002), little settlement by this family may be an indicator of stress. In contrast, the proportion of Poritidae (18%) on the settlement tiles of Reunion was higher than this reported on the Great Barrier Reef (Wallace 1985c; Fisk & Harriott 1990) and in Kenya (Franklin et al. 1998; Mangubhai et al. 2007). In Reunion, the eutrophication of reefs has caused a shift in coral populations previously dominated by the senstitive *Acropora* spp. and now by the more resistant *Porites* spp. and *Montipora* spp. (Naim 2006; Bigot 2008). The high settlement rate observed in Poritidae may reflect the increasing dominance of this family on the Reunion reefs.

4. Influence of thermal stress on the coral early-life

Testing the influence of thermal stress on the coral early-life was a technical challenge as working on coral larvae and juvenile polyp is something new (Petersen 2005; Guest et al. 2013). Unfortunately this experiment could be conducted only in South Africa as the study species were not observed spawning in Reunion. In addition, the juvenile development was obtained in *P. daedalea* only for technical reasons.

4.1.Larval development

The temperature treatments significantly affected the larval and juvenile development in the study species, although *P. daedalea* appeared to be more resistant to thermal stress than *A. austera*. During the larval development, a slight increase of 2°C above ambient (28°C) diminished the survival of *A. austera* larvae compared to the control treatment (26°C). This was nevertheless compensated by a faster rate of larval development. This response to

increase temperature was also observed in several other species (Nozawa & Harrison 2000; Edmunds et al. 2001; Nozawa & Harrison 2007; Randall & Szmant 2009b; Heyward & Negri 2010). O'Conner and co-authors (2007) demonstrated that a slight rise in temperature tend to increase metabolism which in return enhanced the larval development. In *P. daedalea,* the increase in temperature at 2 and 4°C above ambient (at 28 and 30°C respectively) had no effect on the rate of larval development. The survival of larvae was diminished in the warmer treatment (30°C) but remained similar between the slightly heated (28°C) and the control treatment (26°C). Other Favidae coral such as *Favites abdita, F. chinensis* of Japan (26°N) showed similar resistance to high temperature (1-5°C above 27°C, Negri et al. 2007), suggesting that the larvae of this family may be more resistant to thermal stress than other coral families. Nevertheless, it was not the case in the Favidae *Diploria strigosa* of the Caribbean since impaired larval development was reported from temperatures of 1-3°C above ambient (29°C, Bassim et al. 2002; Bassim & Sammarco 2003).

4.2.Settlement

In *P. daedalea*, the temperature had little influence on the timing of settlement of larvae that was concentrated in a peak at 15 days after spawning (DAS). The proportion of settled corals was nevertheless lower in the elevated temperature treatments (14 and 23% at 28 and 30°C respectively) than in the control (25%). New settlement in *P. daedalea* of South Africa was observed up to three months after spawning (100 DAS), while it stopped at 70 DAS in the heated treatments (28 and 30°C). A similar extended period of settlement was observed in *P. daedalea* of the Great barrier reef (93-124 DAS, Nozawa & Harrison 2000). The long pre-competency period and delayed settlement observed in *P. daedalea* in South Africa and GBR is likely to contribute to the broad biogeographical range distribution of this species in the Indo-pacific regions (Nozawa & Harrison 2000). Nevertheless, temperature increase in the context of global warming may affect the mesoscale dispersal of larvae in this species.

4.3.Juvenile development

High temperature (4°C above ambient) significantly affected the rate of development, the concentration of zooxanthellae and the survival in juvenile polyp of *P. daedalea* in South Africa (Table 29). In contrast, there was no significant difference in these parameters between the control and the slightly heated treatments (2°C above ambient). These results suggest that *P. daedalea* juveniles may tolerate a slight increase in sea temperature by 2°C, but their upper

thermal limit of in South Africa is probably close to 30°C. Most recruit of the warmest treatment (30°C) remained completely bleached for the five months of experiment and showed halted development and low survival. From three months after spawning, 5% of the recruits showed a sudden increase in zooxanthellae concentration and was accompanied by a fast polyp development. At the end of the experiment, a total of 17 recruits over the 166 that settled in this treatment, reached a similar development stage that in the control treatment suggesting that they may have acclimatised to the warm conditions. This may have been possible thought the acquisition of thermal-tolerant zooxanthellae (Rowan 2004; Berkelmans & van Oppen 2006; Ladner et al. 2012) or to the expression of a heat stress genes in some genetically diverse recruits (Meyer et al. 2009; Voolstra et al. 2009; Polato et al. 2010).

5. Management perspectives

5.1.South Africa: a 'temperature refuge' for corals?

In theory, the South African reefs seemed to be good candidates to become "temperature refuges" for coral in the Western Indian Ocean as they are exposed to lower temperature regimes (yearly average of 24°C) than the tropical and equatorial reefs of the Region. This idea is corroborated by the fact that the severe increase in seawater temperature during the 1998 ENSO event had no effect on the South African corals (Wilkinson 2000), while it resulted in 80-95% mortality of corals at the most heavily impacted sites (e.g. Seychelles, Maldives, India, Wilkinson 2000). Furthermore our results showed that active sexual reproduction in coral is encountered on the South African reefs, characterised by high polyp fecundity and an important larval supply. If the reefs remained in the current health status as they are, they could at as a coral reservoir in the near future.

Nevertheless the South African reefs will not be saved from the temperature increase in the context of global warming. An average increase of +0.08°C yr^{-1} is currently observed on the South African reefs (1994-2006, Schleyer et al. 2008b). If this trend is maintained in the coming years, the mean seawater temperature will be 2°C warmer in 2030 and 4°C warmer by 2060 than today's level. Our experimentations showed that the embryos and larvae of the South African *Acropora austera* and *Platygyra daedalea* were sensitive to elevated temperature, particularly during embryogenesis. In *A. austera* the increase in temperature induce a faster larval development. The diminished pre-competency period in larvae under high temperature may have strong consequences for larval dispersal as this may favour the

local settlement of larvae and lead to greater genetic isolation of reefs. The upper limit of thermal stress in *P. daedalea* proved to be higher than in *A. austera* i.e. around 30°C. Nevertheless this threshold may be reached rapidly in the context if global warming.

Our results showed that the study species may have acclimatised to the lower temperature regime on the South African reefs and became probably more sensitive to increase in temperature than their tropical counterparts. The bleaching threshold in South African corals (28-29°C, Celliers & Schleyer 2002) is lower than on this reported on tropical reefs (30-32°C, Fitt et al. 2001) confirming the increase coral sensitivity to elevated temperature. This study tend to corroborate the hypothesis proposed by Schleyer & Celliers (2003) that changes on South African reefs could precede those on more tropical reefs. If this sensitivity to temperature is verified in other species, the roles of 'temperature refuge' and 'coral reservoir' will be uncertain in South Africa. Furthermore, the other impacts of climate change such as ocean acidification were not investigated in this study. They may drive different pattern in the coral response to climate change. The capacity of coral to adapt to the rapidly changing conditions is unknown. Nevertheless this study has shown a possible acclimitisation in some *P. daedalea* juveniles after 5 months of exposure to temperature up to 4°C above ambient, suggesting that they may be unexpected responses of corals to elevated temperatures. If the South African reefs are maintained under low levels of disturbance and in their relative good health index, there may be additional time for acclimatisation and adaptation of the local corals in the rapidly changing environment.

5.2.Need for reinforced reef protection in Reunion

In Reunion, the anthropogenic disturbances on the reefs seem to strongly affect the coral reproduction and recruitment. A lower polyp fecundity and diminished recruitment rate were found in Reunion compared to South Africa or other tropical reefs. Moreover the taxonomic composition of the recruit on tile seemed to reflect the shift in coral community observed from the 80's that is the direct consequence of anthropogenic disturbance (Naim 1993; Naim et al. 1997; Bigot 2008; Tourrand et al. 2013).

On the reef flat, which is strongly exposed to coastal development and water pollution, the anthropogenic pressures have favoured the proliferation of cyanobacteia and algae, which outcompete with coral for light and lead to algae dominance in certain area (Cuet et al. 1988; Naim et al. 1997). Theses effect may be weaker on the reef slope as they may be diluted by

the large volume of oceanic water that arrives on the reef (Cuet et al. 2011). Nevertheless riverine output and ground water discharge may reject enriched water on the reef slope (Joint, pers com.). The long-term monitoring of the reef slope using the GCRMN method (Global Coral Reef Monitoring Network) indicate a decrease in coral cover and shift in coral composition on reefs slopes of Reunion(Bigot 2008).

The anthropogenic disturbances observed on the Reunion reefs may exacerbate the impact of climate change and limit the coral capacity to resist or adapt to changes. There is therefore an urgent need to reduce the human impacts on the Reunion reefs. The Marine Reserve established from 2007 may be one of the solutions. Despite its young age and its relative small size, some positive effects of the reef protection under the Marine Reserve are already visible (Chabanet et al. Point 1 de la reserve). In addition, settlement tiles were deployed in no-take and exploited reef-flat areas of the Marine Reserve of Reunion in parallel with this study. A significantly higher settlement rate was observed in the no-take than in the exploited zones of St Leu, suggesting that the reduction of the human pressure may favour the replenishment of damaged areas (Fig. 3). This trend was not observed in la Saline (Fig. 3) where eutrophication remained the major issue. The no-take area may have therefore little influence on coral recruitment in la Saline. Other protective measures should be applied, such as the reduction of urban and agricultural water pollution.

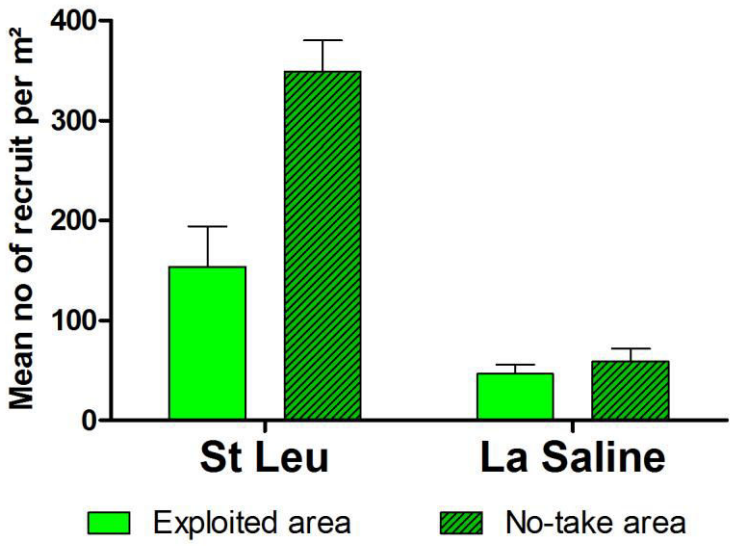


Figure 48: Annual recruitment rate on the reef-flat of St Leu and La Saline.

6. Directions for future research

6.1. Broaden the scope of the study

This study investigated the coral reproduction, fecundity and sensitivity to elevated temperature in two species on two different reef systems in the Indian Ocean. It required to be extended to other species and other localities to verify the trend observed in our results. In addition, only the effect of temperature on the early-life of corals was tested. Nevertheless other impacts associated with climate change, such as ocean acidification, need to be addressed. They may act in synergy with the temperature effects and drive different coral responses.

Our study aimed at gaining a comprehensive picture of the coral life-cycle from gameteogenesis to larval settlement and juvenile development. Juvenile development was observed in aquarium; nevertheless, no comparative data were obtained from in-situ measurements on the reefs. Furthermore the study of recruitment on the tiles deployed on the reefs allowed to assess for larval settlement but did not inform on the post-settlement mortality. For example, it is possible that few recruits survived on the marginal reefs of South Africa after settlement despite a supply of larvae. The count of juvenile and adult corals along transects as described in Penin and co-authors (2007) may provide estimates of the recruit survivals in the years following settlement.

6.2. Protocol for the rapid assessment of coral fecundity

Fecundity may be impaired by several forms of stress and is usually more sensitive than other measures of performance such as growth and respiration (Begon et al., 1986). Indeed, the lower fecundity reported in the corals of Reunion was probably the result of the chronic disturbances reported on the reefs, while corals in South Africa are exposed to more pristine conditions. The number and size of oocytes per polyp may therefore be a relevant index of the coral reproductive status on a given reef. Nevertheless, the estimation of polyp fecundity required some initial knowledge on the coral breeding seasonality. Histology analyses are the most adequate method to study precisely the gamete development in corals and detect the occurrence of overlapping gametogenic cycles. It is, however, a time-consuming method that required equipment and repetitive samplings.

Further research should therefore focus on developing a simple protocol to estimate the coral reproduction status and fecundity. For example, if the timing of spawning is known, the coral sampling could be concentrated around this event. In the Western Indian Ocean, little information on coral reproductive mode and date of spawning is available despite the repetitive observations of coral spawning on reefs like in Mauritius or Seychelles (Montoya-Maya pers com.). It will be therefore interesting to setup a network of people in the Region to gather all the knowledge on the timing of coral spawning in the Region.

6.3. Using genetic to determine the origin and identity of coral recruit on tiles

One limiting aspect of the recruitment study on tiles is that the coral spats can mainly be identified at the family level. Furthermore, the origin of spats remained unknown while there is a growing need to assess whether the larval supply is localised or originates from distant populations. The recruits settling on tile provide interesting and easily available materials for use in genetic studies that may help to investigate coral connectivity. To date most genetic studies are focusing on juvenile or adult colonies. Therefore, they do not take into account the genetic diversity of all the migrants arriving on reefs that may have not survived after settlement.

6.4. Development of coral breeding techniques in aquarium

The development of successful breeding techniques for coral larvae and juveniles issued from sexual reproduction is very recent (Petersen 2005; Guest et al. 2013) and crucial data on the early-life in corals are still missing (e.g. settlement behaviour, survival, zooxanthelllae acquisition).These techniques have prospect for reef restoration as they allow to cultivate e*n masse* genetically diverse corals. In addition, the breeding of sexual recruits in control environments provide an opportunity to assess for the effect of environmental disturbances separately or in combinations. In this study, we obtained only a limited settlement of *A. austera* (5%), while more than 48% of the *P. daedalea* larvae settled in aquarium. Further collaboration between scientists and aquarists is required to adjust the coral breeding techniques and breed sensitive coral species over long period of time.

In addition, there is a crucial lack of knowledge on adequate settlement surface for coral settlement while this step is crucial for reef recovery and replenishment. Mainly, three coral families (Pocilloporidae, Acroporidae and Poritidae) settled on the tiles deployed on the reef

during recruitment surveys. This may, therefore, represent an underestimation of the coral recruitment rate and diversity. For example, Favidae are rarely encountered on settlement tiles while our study has shown successful larval settlement and juvenile polyp development in *P. daedalea* in aquarium. The study of settlement in aquarium may therefore help to assess the factors that control this process in order to develop a substratum better adapted for recruitment surveys on the reefs.

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